B. Claim of Priority and Related Applications

The Action notes that 37 C.F.R. 1.78 requires a specific reference to prior applications of which the present application claims benefit of priority. Applicants respectfully point out that the preliminary amendment submitted upon filing of the request for continuation, and acknowledged in the Official Communication of December 29, 2000 does provide such a specific reference. However, in the interests of speeding approval of the present claims, Applicants have provided the necessary citations in the enclosed substitute specification.

C. Correction of Drawings

Applicants will forward appropriately corrected drawings at the earliest possible date. In the interim, and for the convenience of the Examiner, Applicants have provided in Appendix E informal copies of FIG. 9, which has been divided and renumbered as FIGS. 9-28, corresponding to the amended Brief Description of the Drawings as provided in the substitute specification.

D. Abstract

Applicants have provided an Abstract in the substitute specification as a separate sheet.

E. Order and Arrangement of the Application

Applicants have ordered the sections of the specification as preferred and provided appropriate section headings in the enclosed substitute specification. Correction of minor errors has also been performed. In particular, the error on page 7, line 19 of the original specification has been corrected (see page 8, paragraph 37 of substitute specification). Further, the Brief Description of the Drawings has been amended to recite appropriate SEQ ID NO: listings as required by the Action. No new matter has been introduced through these amendments.

F. Claims 14-34 are enabled under 35 U.S.C. §112, First Paragraph.

The Action has rejected claims 14-34 under 35 U.S.C. §112, first paragraph. The Action asserts that the specification, although enabling for the embodiments exactly disclosed by example, does not enable vaccine compositions and their use in vaccination against any HIV. Applicants respectfully traverse.

First, Applicants respectfully point out that only claims 33 and 34 actually refer to a vaccine, the other claims refer only to polynucleotides. A person skilled in the art would have no difficulty in preparing polynucleotides of the invention using standard cloning procedures. It would therefore appear that the rejection as stated is inappropriate in relation to claims 14 to 32.

With respect to the grounds of rejection, Applicants respectfully note that the invention described in the specification is not directed to a vaccine suitable for prevention or treatment of any particular disease condition. Rather, it describes a general method for formulating a plurality of CTL epitopes so that each CTL epitope can be processed, presented and induce a CTL response (subject to individual HLA restriction). Practical enablement of the formulation concept lies in the induction of CTL responses to a range of CTL epitopes in susceptible animals. As a result, if the problem to solve was an HIV vaccine, one would select from the literature a range of CTL epitopes from appropriate HIV proteins (e.g. *gag, env, nef*, etc) where multiple epitopes from each protein may be chosen to cover a range of HLA restrictions (e.g. A2, B8, A24, B35, etc.).

The total number of CTL epitopes required to formulate an effective vaccine with a wide population coverage may easily exceed 30 or even 40 epitopes. Prior to the instant invention, even though the epitopes themselves existed, there were difficulties involved in formulating these epitopes into vaccines. Applicant has therefore provided a method for formulating multiple CTL epitopes into an effective vaccine. The method is generic as shown by the experimental

Examples, where all appropriately-restricted epitopes induced a response. Selection of appropriate epitopes for any particular application would be within the capabilities of a person skilled in the art.

Furthermore, although it is true that composition claims may be rejected for undue breadth if they read on a significant number of inoperative species, it is not the function of the claims to specifically exclude possibly inoperative substances. *Atlas Powder Co. v. E.I. DuPont de Nemours & Co.*, 224 USPQ 409 (Fed. Cir. 1984). Applicants respectfully submit that the specification enables the construction and use of polytope constructs comprising a plurality of CTL epitopes, including those known from any HIV, even if a few members of the genus claimed may not prove to be highly effective vaccination agents.

The scope of the enabled invention is not limited to the particular CTL epitopes disclosed. The present invention is based on the finding that where two or more CTL epitopes restricted by the same HLA gene are incorporated into a polypeptide construct wherein at least two of the sequences encoding CTL epitopes are contiguous, each lacking their own initiation codon, or spaced apart by non-natural intervening sequences, each epitope is processed efficiently *in vivo* and is capable of generating a primary CTL response. Therefore, following the methods and guidance provided by the present disclosure, the ordinary artisan, through routine experimentation, may determine which particular CTL epitope selection works best for the artisan's intended purpose. Regardless of the polytope construct desired, the artisan is enabled by the present disclosure to make and use a polytope construct as directed by the specification. Such routine experimentation is well within the skill of the ordinary artisan.

That such a polytope may include epitopes from such pathogens as HIV is clearly contemplated and described in the specification. See paragraph 51 of the substitute specification.

The Action points to no scientific reference or principle the sustain the position that if an HIV epitope were chosen to be incorporated into a polytope construct of the present invention it could not successfully be so incorporated and used.

Applicants submit that the instant rejection for lack of enablement is improper. Reconsideration and withdrawal of the rejection is respectfully requested.

G. Claims 14-35 satisfy the written description requirement of 35 U.S.C. §112, First paragraph.

The Action rejects claims 14-35 under 35 U.S.C. §112, first paragraph. The Action alleges that there is not adequate written description provided in the specification to support the conclusion that the Inventors had possession of the claimed invention upon the date of claiming. In particular, the Action correctly notes that there are numerous CTL epitopes available for inclusion in a polytope construct, both where the CTL epitopes are restricted by different or identical HLAs. The Action goes on to conclude that the Applicants have provided an insufficient number of exemplary species to allow adequate description of the claimed genus of CTL epitopes. Applicants respectfully traverse.

The Action argues that the diversity of available CTL epitopes precludes a successful written description but for an exhaustive listing of all possible epitopes, the nucleotide sequences that encode them, and all combinations thereof. Such an exhaustive listing of all possible CTL epitope sequences is not required to demonstrate that the inventors had possession of the invention. That the range of possible CTL epitopes is wide does not preclude adequate description. See *In re Angstadt*, 537 F.2d 498 at 502- 03, 190 USPQ 214, 218 (CCPA 1976) (Applicants "are <u>not</u> required to disclose <u>every</u> species encompassed by their claims even in an unpredictable art.")

Patent law, as set forth by the Federal Circuit and the MPEP, requires only that an "applicant's specification must convey with reasonable clarity to those skilled in the art that, as of the filing date sought, he or she was in possession of the invention. . . ." MPEP 2163 (citing *Vas-Cath, Inc. v. Marhurkar*, 19 U.S.P.Q.2d 1111, 1117 (Fed. Cir. 1991). "Possession" is shown by "such descriptive means as words, structures, figures, diagrams, formulas, etc., that fully set forth the claimed invention." *Lockwood v. Am. Airlines, Inc.*, 107 F.3d 1565, 1572 (Fed. Cir. 1997). Exemplary descriptions provided in the present disclosure include two polytope constructs, each composed of different CTL epitopes. See page 8, Table 1 and page 13, Table 2, which provide a total of 19 distinct CTL epitope sequences.

"Mention of representative compounds encompassed by generic claim language clearly is not required by §112 or any other provision of the statute. But, where no explicit description of a generic invention is to be found in the specification . . . mention of representative compounds may provide an implicit description upon which to base generic claim language." *In re Robins*, 429 F.2d 452, 456-57, 166 USPQ 552, 555 (CCPA 1970).

CTL epitopes are recognized by one of skill in the art of immunology and its related fields, who would understand that the specification of particular epitopes does not limit the scope of the disclosed invention. Applicants also respectfully point out that the CTL epitopes of the claims are not "from different HLA alleles" but rather are restricted by different classes of HLA. The epitopes themselves are selected from whatever pathogen or tumor against which one desires to make a vaccine. Applicants draw attention to Hobohm and Meyerhans, (1993), (a copy of which is enclosed) which describes a search method for identifying anchor residues in T cell epitopes. To wit, the abstract of this article states that "[t]his method can be used to predict the natural short epitope inside longer antigenic peptides and to predict the epitopes anchor

residues." And, "[a] large set of MHC class I-restricted peptides has been described." Examples of T cell epitope sequences known to the art as of 1993 are set out in Table 3 of the reference.

Applicants also draw attention to Rammensee *et al.*, (1995) as indicative of the level of knowledge in the relevant art. The article was written in 1994 (see page 178, first paragraph) and lists "a couple of hundred" MHC (HLA) ligands. This single reference therefore provides numerous examples of epitope sequences known to the art at the time of invention.

Furthermore, the Action itself refers to numerous publications that disclose a large number of CTL epitopes (see the Action, pages 8-15). These references establish that a large number of CTL epitopes was already known and identified as "CTL epitopes" in the literature available to the ordinary artisan of immunology and related fields as of the filing date of the present invention. It would have been well within the skill of a person working in the relevant field to select appropriate CTL epitopes for use in recombinant nucleic acid vaccines against any infectious agent, or indeed, against HIV. As mentioned above, the selection of a particular CTL epitope sequence is not limiting. Therefore, the disclosure provides sufficient description of the method of constructing and using the polytopes of the invention, regardless of the specific epitope sequences chosen.

The situation presented in *Eli Lilly*, cited by the examiner, is inapposite in that the claims at issue in *Lilly* did not reference a suite of specific, known molecules as illustrative of the genus claimed. The claims in Lilly encompassed species of cDNA as yet undetermined. The inventors here have provided working examples, and a further list of exemplary CTL epitopes and sources of epitopes known to and within the reach of the relevant artisan at the time of filing. Applicants respectfully submit that they have described more than a sufficient number of representatives of

the genus of CTL epitopes within the context of the methods of the invention so as to demonstrate that they have fully set forth and possess the invention. Applicants respectfully request that the rejection be withdrawn.

H. No new matter is introduced by claims 14-34.

The Action rejects claims 14-34 under 35 U.S.C. §112, first paragraph. The Action argues that claims 14-34 "represent a departure from the specification and the claims as originally filed" and as such, introduce new matter. In particular, the Action notes that the disclosure provides that "at least one recombinant protein is 'substantially free of sequences encoding peptide sequence naturally found to flank the CTL epitopes,'" and concludes that the current claims introduce new matter in view of this disclosure. Applicants respectfully traverse.

Applicants respectfully submit that the Action does not clearly point out how the claims depart substantially from the content of the specification as filed. Assuming the Action intends to draw attention to the distinction between a recombinant protein and the polynucleotides encoding such recombinant proteins, Applicants respectfully point out that there is clear support for such claims throughout the specification, but most particularly at page 2, lines 10-16. Applicants note that the feature that the recombinant polynucleotide is "substantially free of sequences encoding peptide sequence naturally found to flank the CTL epitopes" is a preferred embodiment of the present invention.

Further, the specification as filed provides support for a "plurality" of epitopes. See, for example, page 2, paragraph 14 and page 2, paragraph 6. Applicants respectfully submit that these passages provide ample support for at least two CTL epitopes. The passage at page 3, lines 28-29 provides support for at least ten (or more) epitopes. The specification additionally

provides support for a polytope construct comprising multiple epitopes that are restricted by the same HLA allele at page 3, paragraph 14 and page 13, paragraph 50.

The specification also provides support for the viral vector of claim 21. See, *e.g.*, page 3, paragraph 13; page 4, paragraph 18; page 9, paragraph 38 of the specification.

Applicants respectfully request reconsideration and withdrawal of this rejection.

I. Claims 20-24 are definite under the second paragraph of 35 U.S.C. §112.

The Action rejects claims 20-24 under 35 U.S.C. §112, second paragraph as indefinite because their antecedent basis is improper. Present claims 20-24 more clearly point out the invention. Specifically, claim 20 now more clearly recites a vector, (See, *e.g.*, page 3, paragraph 13; page 4, paragraph 18; page 9, paragraph 38 of the specification) to which claims 21-24 properly refer. Applicants believe these amendments render the rejected claims definite and respectfully request withdrawal of the rejection.

The Action rejects claims 21 and 24 as ambiguous, in that they are said to refer to "virus like-particles" or VLP, which the Action alleges may refer at once to either viral particles or DNA plasmid from naturally occurring virus. Applicants respectfully point out that the term "virus-like particle" is a well-known term of art. See, for example, the references cited at page 3, paragraph 13. As such, the term is unambiguous to one of skill in the art. Therefore, claims 21 and 24 are themselves not ambiguous. Applicants respectfully submit that the present claims 21 and 24, which refer to the vector being a virus-like particle, are clear. Applicants therefore respectfully request that the rejection be withdrawn.

J. Claims 14-16, 20-22, 25, 33 and 34 are novel under 35 U.S.C. §102(b) over Whitton *et al.*

The Action rejects claims 14-16, 20-22, 25, 33 and 34 as anticipated by Whitton *et al.* (1993). Applicants respectfully traverse.

The Action cites Whitton *et al.* as disclosing all of the claimed limitations of the present invention. However, the present invention expressly recites a polynucleotide encoding a plurality of CTL epitopes wherein "at least two of the sequences encoding said CTL epitopes are contiguous or spaced apart by intervening sequences, wherein said intervening sequences do not (i) comprise and initiation codon or (ii) encode naturally occurring flanking sequences of the epitopes. Whitton *et al.* does not disclose such a polynucleotide construct.

Among the several important and unexpected discoveries embodied in the present invention is that CTL epitopes can be joined end to end either contiguously or with spacing amino acids not normally associated with those epitopes. The joining thus may expressly exclude intervening initiation (or start) codons. Despite the omission of these, or other naturally occurring flanking sequences, the resulting polypeptide, when formulated appropriately, can be processed within the cytoplasm of an antigen presenting cell (APC) so that the identity of the individual CTL epitopes is preserved. The induction of an *in vivo* CTL response to these epitopes is proof of the preservation of their identity.

Whitton *et al.* at best provides for a construct containing two epitopes, each with a start codon. See Figure 1 of Whitton, page 349. Whitton refers to these constructs as "mini-genes" precisely because they each contain their own start codon for initiation of translation. As is clear from the present claims, however, the inclusion of a start codon for every epitope of the present invention is excluded. Therefore, Applicants respectfully submit that the invention as claimed is

not anticipated by Whitton *et al.* (1993) because all of the claimed limitations are not contained within the reference. Applicants request the rejection be withdrawn.

K. Claims 14-16, 20-22, 25, 27, 33 and 34 are novel under 35 U.S.C. §102(b) over Lawson *et al.*

The Action rejects claims 14-16, 20-22, 25, 27, 33 and 34 as anticipated by Lawson *et al.* (1993). Applicants respectfully traverse. Lawson *et al.* disclose ***not two*** CTL epitopes present in a single construct, ***but one epitope***, the NP epitope, accompanied by the signal sequence of the adenovirus E3/19K glycoprotein. See Lawson at page 3506 under the heading "Viruses." Lawson *et al.* does not discuss this signal sequence, (designated ES) as a CTL epitope. Indeed, the focus of the experiments disclosed in Lawson is on the NP epitope and comparisons to results of vaccinations with the full length NP. Thus, absent any indication that the ES sequence functions as a CTL epitope, the constructs of Lawson *et al.* do not contain two or more CTL epitopes. Lawson *et al.* cannot anticipate the present claims, which have as an express limitation that at least two epitope encoding sequences are present in the polytope construct. Applicants respectfully request the withdrawal of the application.

L. Claims 14 and 17-19 are not obvious under 35 U.S.C. §103(a) over Whitton *et al.*

The action rejects claims 14 and 17-19 as unpatentable over Whitton *et al.* under 35 U.S.C. §103(a). Applicants respectfully traverse the rejections.

Whitton discloses the epitopes as "short open reading frames". See page 349, lines 8 to 11 of Whitton. Whitton refers throughout to "minigenes." As used by Whitton, minigenes are separate entities that are expressed separately. These minigenes are identified by the presence of an initiation codon at the start of translation for each of the two epitopes. The nucleotide constructs of the present invention differ from the minigenes disclosed in Whitton *et al.* because

the sequences encoding the minimal CTL epitopes are such that the epitopes are contiguous or spaced apart by intervening sequences which do not comprise an initiation codon and which are not naturally occurring flanking sequences, *i.e.* not sequences that occur naturally outside of the minimal epitope sequence.

This is a significant and non-obvious difference. The present invention is based on the surprising finding that minimal epitopes can be linked together without naturally occurring flanking sequences (including start sites) yet still be correctly processed. This is not suggested by Whitton *et al.* In fact, Whitton teaches the presentation of the epitopes in the form of "minigenes" to achieve correct processing and therefore teaches away from the present invention. Whitton does not provide any teaching or motivation to the skilled person to express multiple epitopes in any form other than as "minigenes," *i.e.* as separate short open reading frames with accompanying start codons, regardless of the number of epitopes encoded.

Since Whitton actually teaches away from the present invention, Applicants respectfully submit that the present invention is therefore patentable under 35 U.S.C. §103(a) over Whitton *et al.* and request that the rejections be withdrawn.

M. Claims 14, 17- 21, and 23-32 are not obvious under 35 U.S.C. §103(a) over Whitton *et al.* or Lawson *et al.* in view of Berzofsky *et al.*, Burrows *et al.*, Del Val *et al.*, Latron *et al.*, Panicali *et al.*, Adams *et al.*, Celis *et al.*, Widman *et al.*, or Potter *et al.*

The Action rejects claims 14, 17- 21, and 23-32 under 35 U.S.C. §103(a) in view of Whitton *et al.* or Lawon *et al.* in combination with any of the above captioned references. Applicants respectfully traverse.

Neither the Whitton or Lawson references alone or in combination are sufficient to render the present invention unpatentable under 35 U.S.C. §103(a). The deficiencies of the Whitton

reference are discussed above. Likewise, the deficiencies of the Lawson reference are also discussed above. Additionally, Lawson cannot be read by one of skill in the art to suggest that the disclosure of Whitton be modified to remove the start codons from the di-epitope constructs disclosed therein. The focus and language of Lawson does not address that issue at all.

Therefore, there must be some suggestion or motive provided by the balance of the cited references or in the knowledge of the art to modify the disclosure of Whitton *et al.* or Lawson *et al.* to make the claimed invention. However, no such suggestion is to be found in the balance of the cited references. None of the references address the central issue of the invention -- whether the presence or absence of the naturally occurring intervening sequences such as the start codon would effect the processing and effectiveness of a polyepitope construct. Of course, no reasonable expectation of success can be construed from references that do not even suggest the appropriate elements of the present invention.

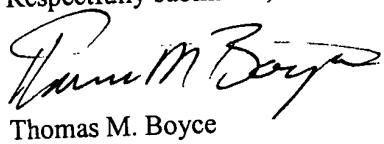
Since any such suggestion must be accompanied by a reasonable expectation of success, and all the claimed limitations must be found within the cited references (*In re Vaeck*, 947 F.2d 488, 20 USPQ2d 1438 (Fed. Cir. 1991) and MPEP §2143) Applicants respectfully request the rejections be withdrawn. Neither Whitton *et al.* nor Lawson *et al.*, nor any of the other cited references contain or suggest all the claimed limitations of the invention. Perforce, they cannot render the invention obvious.

N. Conclusion

In view of the above, Applicants respectfully submit that the claims are in condition for allowance. Applicants respectfully and earnestly request notification to that effect. The

Examiner is invited to contact the undersigned attorney at (512) 536-3043 with any questions, comments or suggestions relating to the referenced patent application.

Respectfully submitted,



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APPENDIX C

CLAIMS MARKED FOR AMENDMENT IN APPLICATION SN 09/576,101

14. A polynucleotide comprising a nucleic acid sequence encoding a plurality of CTL epitopes, wherein at least two of the sequences encoding said CTL epitopes are contiguous or spaced apart by intervening sequences, wherein said intervening sequences do not (i) comprise an initiation codon or (ii) encode naturally occurring flanking sequences of the epitopes.

15. A polynucleotide comprising a nucleic acid sequence encoding a plurality of CTL epitopes, wherein the sequence encoding said CTL epitopes are contiguous.

16. The polynucleotide of claim 14, wherein said polynucleotide encodes two CTL epitopes.

17. The polynucleotide of claim 14, wherein said polynucleotide encodes three CTL epitopes.

18. The polynucleotide of claim 14, wherein said polynucleotide encodes nine CTL epitopes.

19. The polynucleotide of claim 14, wherein said polynucleotide encodes ten CTL epitopes.

20. (Amended) [The]A vector comprising the polynucleotide of claim 14[, further defined as an expression vector].

21. (Amended) The [polynucleotide]vector of claim 20, wherein said vector is selected from the group consisting of a viral vector and a virus-like particle (VLP).

22. (Amended) The [polynucleotide]vector of claim 21, wherein said viral vector is a vaccinia vector.

23. (Amended) The [polynucleotide]vector of claim 21, wherein said viral vector is an avipox virus vector.

24. (Amended) The [polynucleotide]vector of claim 21, wherein said vector is a VLP.

25. The polynucleotide of claim 14, wherein at least one of said CTL epitopes is derived from a pathogen.

26. The polynucleotide of claim 14, wherein said polynucleotide comprises a nucleic acid sequence encoding CTL epitopes derived from a plurality of pathogens.

27. The polynucleotide of claim 25, wherein said pathogen is selected from the group consisting of Epstein Barr Virus, Influenza Virus, Cytomegalovirus, Adenovirus and HIV.

28. The polynucleotide of claim 14, wherein at least one of said epitopes is derived from a tumor protein.

29. The polynucleotide of claim 14, further comprising a nucleic acid sequence encoding a T helper cell epitope, a B cell epitope, or a toxin.

30. The polynucleotide of claim 14, further comprising a nucleic acid sequence encoding a T helper cell epitope.

31. The polynucleotide of claim 14, further comprising a nucleic acid sequence encoding a B cell epitope.

32. The polynucleotide of claim 14, further comprising a nucleic acid sequence encoding a toxin.

33. A nucleic acid vaccine comprising a polynucleotide comprising a nucleic acid sequence encoding a plurality of CTL epitopes, wherein at least two of the sequences encoding said CTL epitopes are contiguous or spaced apart by intervening sequences, wherein said intervening sequences do not (i) comprise an initiation codon or (ii) encode naturally occurring flanking sequences of the epitopes, and an acceptable carrier.

34. A nucleic acid vaccine comprising a polynucleotide comprising a nucleic acid sequence encoding a plurality of CTL epitopes, wherein the sequences encoding said CTL epitopes are contiguous, and an acceptable carrier.

~~POLYEPITOPE VACCINES~~POLYEPITOPE VACCINES

1. This application is a continuation of co-pending application Serial No. 08/776,337 filed April 21, 1997, which is a nationalization under 35 U.S.C. §371 of International Application number PCT/AU95/00461 filed July 27, 1995, which claims priority to Australian Patent Application number PN1009 filed February 8, 1995, and Australian Patent Application number PM7079 filed July 27, 1994.

BACKGROUND OF THE INVENTION

1. Field of the Invention

2. The present invention relates to vaccines containing a plurality of cytotoxic T lymphocyte epitopes and to polynucleotides including sequences encoding a plurality of cytotoxic T lymphocyte epitopes.

2. Description of the Related Art

3. CD8 + αβ cytotoxic T lymphocytes (CTL) ~~recognise~~recognize short peptides (epitopes, usually 8-10 amino acids long) associated with specific alleles of the class I major histocompatability complex[1] (MHC). The peptide epitopes are mainly generated from cytostolic proteins by proteolysis, a process believed to involve the multicatalytic proteosome complex[2-7]. Peptides of appropriate length are transported into the endoplasmic reticulum where specific epitopes associate with MHC. The MHC/epitope complex is then transported to the cell surface for recognition by CTL. The influence of sequences flanking CTL epitopes on the proteolytic processing of these epitopes remains controversial[8-12]. However, by constructing a recombinant vaccinia coding for an artificial polypeptide protein containing nine CD8 CTL epitopes in sequence, the present inventors have determined that the natural flanking sequences of CTL epitopes are not required for class I processing, that is each epitope within the polyepitope protein was always efficiently processed and presented to appropriate CTL clones by autologous polyepitope vaccinia infected target cells.

Summary of the Invention

4. Accordingly, in a first aspect, the present invention consists in a recombinant polyepitope cytotoxic T lymphocyte vaccine, the vaccine comprising at least one recombinant protein including a plurality of cytotoxic T lymphocyte epitopes from one or more pathogens, wherein the at least one recombinant protein is substantially free of sequences naturally found to flank the cytotoxic T lymphocyte epitopes.

5. Preferably, the at least one recombinant protein does not include any sequences naturally found to flank the cytotoxic T lymphocyte epitopes. However, it should be understood that small lengths (e.g. 1-5 amino acids) of sequences naturally found to flank the cytotoxic T lymphocyte epitopes may be included. The phrase "substantially free of sequences naturally found to flank the cytotoxic T lymphocyte epitopes" is to be taken as including such short lengths of flanking sequences.

6. In a second aspect, the present invention consists in a polynucleotide, the polynucleotide including at least one sequence encoding a plurality of cytotoxic T lymphocyte epitopes from one or more pathogens, and wherein the at least one sequence is substantially free of sequences encoding peptide sequences naturally found to flank the cytotoxic T lymphocyte epitopes.

7. Again, it is to be understood that "substantially free of sequences encoding peptide sequences naturally found to flank the cytotoxic T lymphocyte epitopes" includes the possibility of including short peptide (e.g 1-5 amino acids) sequences naturally found to flank the cytotoxic T lymphocyte epitopes.

8. In a third aspect, the present invention consists in a nucleic acid vaccine, the vaccine comprising the polynucleotide of the second aspect of the present invention and an acceptable carrier.

9. In a fourth aspect, the present invention consists in a vaccine formulation, the vaccine comprising the recombinant protein of the first aspect of the present invention and an acceptable carrier and/or adjuvant.

10. In a preferred embodiment of the present invention the at least one recombinant protein includes, and the at least one sequence encodes, at least three cytotoxic T lymphocyte epitopes.

11. In a further preferred embodiment, the epitopes are from multiple pathogens.

12. It is also envisaged that the vaccines according to the invention may include immunomodulatory compounds (such as cytokines), other proteins/compounds (such as melittin or regulatory proteins) and/or adjuvants. The vaccines may also include helper epitopes/CD4 epitopes and proteins, B-cell epitopes or proteins containing such epitopes, for example tetanus toxoid. Another example of a vaccine according to the invention comprises a recombinant vaccine construct wherein the polytope including the CTL epitopes is linked to an extracellular glycoprotein or glycoproteins containing B-cell and/or CD4 epitopes.

13. The vaccines according to the invention may be delivered by various vectors, for example vaccinia vectors, avipox virus vectors, bacterial vectors, virus-like particles (VLP's) and rhabdovirus vectors or by nucleic acid vaccination technology. As polytope proteins are likely to be sensitive to proteolysis during manufacture and/or serum following injection, we envisage that such vaccines may best be delivered using nucleic acid vaccination technologies[12], vector systems or adjuvant systems which protect the polytope protein from proteolysis. Additional information regarding vectors may be found in Chatfield *et al*, Vaccine 7, 495-498, 1989; Taylor *et al*, Vaccine 13, 539-549, 1995; Hodgson "Bacterial Vaccine Vectors" in Vaccines in Agriculture.

14. A polytope vaccine according to the invention may also include a large number of epitopes (e.g. up to 10 or more) from one pathogen so that the HLA diversity of the target population is covered. For instance a vaccine containing epitopes restricted by HLA A1, A2, A3, A11 and A24 would cover about 90% of the Caucasian population.

15. A polytope vaccine according to the invention may also be constructed such that the multiple epitopes are restricted by a single HLA allele.

16. In a preferred embodiment of the fourth aspect of the present invention the vaccine formulation includes ISCOMs. Information regarding ISCOMs can be found in Australian patent No 558258, EP 019942, US4578269 and US4744983, the disclosures of which are incorporated herein by reference.

17. In order that the nature of the present invention may be more clearly understood preferred forms thereof will now be described with reference to the following examples and accompanying ~~Figures in which:~~ Figures.

Brief Description of the Drawings

~~Figure~~18. FIG. 1. Construction of a recombinant vaccinia that expresses a synthetic DNA insert coding for the polytope protein (SEQ ID NO:10), which contains nine CTL epitopes in sequence. Boxed sequences representing linear B cell epitopes.

~~Figure~~19. FIG. 2. CTL recognition of each epitope expressed in the recombinant polytope vaccinia construct of FIG. 1.

~~Figure~~20. FIG. 3. Polytope vaccinia can recall epitope specific responses. Bulk effectors from donors

(A) CM - A24, A11, B7, B44; (B) YW - A2, B8, B38 ~~and~~

and (C) NB - A2, A24, B7, B35 were generated by infecting peripheral blood mononuclear cells (PBMC) with the polytope vaccinia of FIG. 1., SEQ ID NO: 10, at a MOI of 0.01 for 2 hours followed by 2 washes. After 10 days culture in 10% FCS/1640 RPMI the bulk effectors were used against autologous phytohaemagglutinin T cell blasts target cells (E:T 20:1) ~~sensitised~~sensitized with the indicated peptide (10μM) in a standard 5 hour chromium release assay[14]. Peptides used were (A) SEQ ID NO: 6, SEQ ID NO: 4, SEQ ID NO: 9, SEQ ID NO: 8; (B) SEQ ID NO: 6, SEQ ID NO: 11, SEQ ID NO: 4, SEQ ID NO: 17; and (C) SEQ ID NO: 6 and SEQ ID NO: 21. Bulk effectors generated by the addition of irradiated autologous A type LCLs[14] (LCL to PBMC ratio 1:50) gave similar results to that shown above.

~~Figure 4 shows the construction~~21. FIG. 4. Construction of a polytope DNA insert including ten murine CTL epitopes as in Table 2.

~~Figure 5 shows the sequence~~22. FIG. 5. Polypeptide sequence (SEQ ID NO: 22) of the polytope DNA insert ~~of Figure 1.~~(SEQ ID NO: 23) of FIG. 4.

~~Figure 6 provides results~~23. FIG. 6A. Results of CTL assays conducted on splenocytes harvested from BALB/c mice vaccinated with recombinant vaccinia including the DNA insert of ~~Figure 3.~~

FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK- vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 19.

24. FIG. 6B. Results of CTL assays conducted on splenocytes harvested from BALB/c mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open

squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 18.

25. FIG. 6C. Results of CTL assays conducted on splenocytes harvested from BALB/c mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 20.

26. FIG. 6D. Results of CTL assays conducted on splenocytes harvested from BALB/c mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 12.

27. FIG. 6E. Results of CTL assays conducted on splenocytes harvested from CBA mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 13.

28. FIG. 6F. Results of CTL assays conducted on splenocytes harvested from CBA mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 3.

29. FIG. 6G. Results of CTL assays conducted on splenocytes harvested from C57BL/6 mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 1.

30. FIG. 6H. Results of CTL assays conducted on splenocytes harvested from C57BL/6 mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 14.

31. FIG. 6I. Results of CTL assays conducted on splenocytes harvested from C57BL/6 mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 15.

32. FIG. 6J. Results of CTL assays conducted on splenocytes harvested from C57BL/6 mice vaccinated with recombinant vaccinia including the DNA insert of FIG. 5 (open squares), no peptide control (open triangles), or bulk splenocytes from TK-vaccinated mice (open circles) and restimulated with the peptide of SEQ ID NO: 5.

~~Figure 7 shows comparison~~33. FIG. 7. Comparison of spleen MCMV titres (± standard error) 5 weeks after polytope vaccinia vaccination and 4 days after MCMV challenge. P values - unpaired ~~student~~Student's t-test

~~Figure 8~~34. FIG. 8. DNA vaccination with different plasmids in ~~Balb/c~~BALB/c mice.

~~Figure 9. Murine Polytope vaccinia immunised (IP) mice from these strains (Balb/c, CBA, C56BL/6)~~35. FIGS. 9-28. Lysis of target cells presenting 10 different epitopes by splenocytes from murine polytope vaccinia immunized (IP) mice. Splenocytes from strains BALB/c, (FIGS. 9-20), and C56BL/6 (FIGS. 21-28) had the spleens removed and splenocytes restimulated with the following peptides: FIGS. 9 and 10, ~~peptide (eg for A and A'). effectors were generated by stimulation with influenza NP peptide "TYQRTRALV").~~SEQ ID NO: 19; FIGS. 11 and 12, SEQ ID NO: 18; FIGS. 13 and 14, SEQ ID NO: 20; FIGS. 15 and 16, SEQ ID NO:12; FIGS. 17 and 18, SEQ ID NO:13; FIGS. 19 and 20, SEQ ID NO:3; FIGS. 21 and 22, SEQ ID NO: 1; FIGS. 23 and 24, SEQ ID NO: 14; FIGS. 25 and 26, SEQ ID NO:16; FIGS. 27 and 28, SEQ ID NO:5. The effectors were then used~~on peptide coated targets (A-J), virus infected targets (A'-J') and tumour targets (I').~~in standard CTL assays against peptide-coated target cells, using the same peptide (squares) or no peptide controls (circles in FIGS. 9, 11, 13, 15, 17, 19, 21, 23, 25, and 27), against virus-infected targets (FIGS. 10, 12, 14, 16, 18, 20, 22, 24 and 28), or against tumor targets (FIG. 26). Virus infected targets were either infected~~(A', F'),~~ with allantoic fluid as negative control ~~or murine polytope vaccinia (Vacc Mu PT) (B'-D', F'-J'),~~(FIGS. 10 and 18), with human polytope vaccinia (Vacc ~~Hu~~Nu PT) as the negative control (FIGS. 12, 14, 16, 20, 22, 24 and 28), or the EL-4 line served as a control (FIG. 26).

Description of Preferred Embodiments

Example 1

36. Nine, class I restricted, CTL epitopes from several Epstein-Barr virus nuclear antigens (EBNA) have previously been defined using CTL clones[10, 18-20]. The clones were isolated from a panel of normal healthy Epstein-Barr virus (EBV) seropositive donors and were restricted by different HLA alleles (Table 1). A recombinant polyepitope vaccinia (polytope vaccinia), which coded for a single artificial protein containing all nine of these CTL epitopes, was constructed (see ~~Fig.~~FIG. 1). The DNA sequence coding for this protein was made using splicing by overlap extension (SOEing) and the polymerase chain reaction (PCR) to join six overlapping oligonucleotides. The insert was cloned into pBluescript II, checked by sequencing and transferred into pBCBO7[15] behind a vaccinia promoter to generate pSTPT1. This plasmid was then used to generate the polytope vaccinia virus by marker-rescue recombination[16]. The artificial polytope protein expressed by this vaccinia therefore containing no sequences naturally found to flank the CTL epitopes in their proteins of origin (~~Fig.~~FIG. 1).

~~CTL CLONES~~	~~COGNATE EPITOPES~~	~~SOURCE~~		~~HLA RESTRICTION~~	~~REFS~~
CTL CLONES	COGNATE EPITOPES	SEQ ID NO:	SOURCE	HLA RESTRICTION	REFS
~~LC13~~	~~FLRGRAYGL~~	~~EBNA3~~		~~B8~~	~~13~~
LC13	FLRGRAYGL	6	EBNA3	B8	13
~~LC15~~	~~QAKWRLQTL~~	~~EBNA3~~		~~B8~~	~~14~~
LC15	QAKWRLQTL	11	EBNA3	B8	14
~~CS31~~	~~EENLLDFVRF~~	~~EBNA6~~		~~B44~~	~~15~~
CS31	EENLLDFVRF	4	EBNA6	B44	15
~~YW22~~	~~SVRDRLARL~~	~~EBNA3~~		~~A0203~~	~~14~~
YW22	SVRDRLARL	17	EBNA3	A0203	14
~~CM4~~	~~KEHVIQNAF~~	~~EBNA6~~		~~B44~~	~~13~~
CM4	KEHVIQNAF	9	EBNA6	B44	13
~~NB26~~	~~YPLHEQHGM~~	~~EBNA3~~		~~B3501~~	~~14~~
NB26	YPLHEQHGM	21	EBNA3	B3501	14
~~LX1*~~	~~HLAAQGMAY~~	~~EBNA3~~		~~?~~	~~14~~
LX1*	HLAAQGMAY	7	EBNA3	?	14
~~JSA2~~	~~DTPLIPLTIF~~	~~EBNA3~~		~~B51#/A2~~	~~13~~
JSA2	DTPLIPLTIF	2	EBNA3	B51#/A2	13
~~CM9~~	~~IVTDFSVIK~~	~~EBNA4~~		~~A11~~	~~16~~
CM9	IVTDFSVIK	8	EBNA4	A11	16

Table 1: Description of the CTL clones, their cognate epitopes, the proteins of origin (source) and their HLA restriction. The first two letters of the clones refer to the donors. *Not tested (see ~~Fig.~~FIG. 2). #Recent evidence suggests this epitope may be restricted by HLA-B51. All the epitopes have been ~~minimalised~~minimalized except EENLLDFVRF ~~and DTPLIPLTIF,~~(SEQ ID NO: 4) and DTPLIPLTIF (SEQ ID NO: 2).

37. A DNA sequence coding for the polytope amino acid sequence was designed with codons most frequently used in mammals and incorporated a Kozac sequence[13] and a BamHI site upstream of the start codon. Six 70mer oligonucleotides overlapping by 20 base pairs were spliced together using Splicing by Overlap Extension (SOEing)[14] in 20µl reactions containing standard PCR buffer, 2mM $MgCl_2$, 0.2mM dNTPs, 1.5U of Taq polymerase (hot start at 94°C) using the following thermal program: 94°C for 10 seconds, 45°C for 20 seconds and 72°C for 20 seconds (40 cycles). Half of each gel purified dimer sample was combined in a second PCR

reaction (12 cycles) with the addition of 0.5µl of α^{32}p dCTP. The reaction was run on a 6% acrylamide gel and a slice corresponding to the position of the hexamer product was isolated. Two 20mer oligonucleotides were used to PCR amplify the hexamer using an annealing temperature of 56°C and 25 cycles. The gel purified fragment was cloned into the EcoRV site of pBluescript II KS-, was checked by sequencing and cloned behind the vaccinia P7.5 early/late promoter using the BamHI/SalI sites in the vaccinia plasmid vector pBCBO7[15] to generate pSTPT1. Construction of a TK- recombinant virus was carried out using marker rescue combination between pSTPT1 and VV-WR-L929 as described previously[16]. Plaque purified virus was tested for TK phenotype and for appropriate genome arrangement by Southern blotting of viral DNA[16].

38. To establish whether each epitope could be efficiently processed from the polytope protein the polytope vaccinia was used to infect a panel of target cells, which expressed the HLA alleles restricting each epitope. Autologous CTL clones specific for each epitope were then used as effector cells in standard chromium release assays. In all cases tested the CTL clones ~~recognised~~recognized and killed the HLA matched target cell infected with the polytope vaccinia and the appropriate (see Table 1) EBNA vaccinia (positive controls), but not the TK- vaccinia (negative controls) (~~Fig.~~FIG. 2).

39. Figure 2 shows CTL recognition of each epitope expressed in the polytope vaccinia construct. Effector CTL are listed in Table 1 (E:T ratio 5:1). Target cells (see below) were infected with recombinant vaccinia expressing either (i) the EPV nuclear antigen (EBNA) ~~recognised~~recognized by the CTL clone (see Table 1) (positive control), (ii) TK- (negative control), or (iii) the polytope construct (i.e., Polytope vaccinia). Vaccinia infection of the target cells was at a multiplicity of infection of 5:1 followed by 14-16 hour incubation at 37°C prior to use in the standard, 5 hour, ^{51}Cr-release assay[29]. Clone LX1 was no longer available at the time of assay. Target cells; there are two types of EBV, A and B-type, whose EBNA protein sequences differ significantly. CTL clones LC13, LC15, CM4, NB26, JSA2 and CM9 ~~recognise~~recognize cells transformed with A-type EBV but not B-type EBV, and CTL clones CS31 and YW22 ~~recognise~~recognize cells transformed with A-type EBV and EBV[10,~~18~~ 18-20]. The target cells used for the A-type specific CTL were therefore autologous lymphoblastoid cell lines

transformed with B-type virus (B-type LCLs). The target cell for CS31 and YW22 were EBV negative B cell blasts, established using anti-CD40 antibody and rIL-4[21].

40. An additional series of experiments used the polytope vaccinia to stimulate *in vitro* a secondary CTL response from peripheral blood mononuclear cells (PBMC) obtained from healthy EBV seropositive donors. The resulting bulk CTL cultures were then used as effectors against peptide epitope ~~sensitised~~sensitized autologous PHA blasts in a standard chromium release assay. The polytope vaccinia was capable of recalling CTL responses which were specific for epitopes restricted by the HLA alleles expressed by each donor ~~(Fig.~~(FIG. 3).

41. Figure 3 shows that polytope vaccinia can recall epitope specific responses. Bulk effectors from donors

(A) CM - A24, A11, B7, B44; (B) YW - A2, B8, B38 ~~and~~

and (C) NB - A2, A24, B7, B35 were generated by infecting peripheral blood mononuclear cells (PBMC) with the polytope vaccinia at a MOI of 0.01 for 2 hours followed by 2 washes. After 10 days culture in 10% FCS/1640 RPMI the bulk effectors were used against autologous phytohaemagglutinin T cell blasts target cells (E:T 20:1) ~~sensitised~~sensitized with the indicated peptide (10μM) in a standard 5 hour chromium release assay[19]. Bulk effectors generated by the addition of irradiated autologous A type LCLs[19] (LCL to PBMC ratio 1:50) gave similar results to that shown above.

42. Two linear B cell epitopes ~~(STNS and NNLVSGPEH) recognised~~(STNS, SEQ ID NO: 24 and NNLVSGPEH, SEQ ID NO: 25) recognized by monoclonal antibodies (8G10/48[22] and 8E7/55[23] respectively) were incorporated at each end of the polytope construct ~~(Fig.~~(FIG. 1) to follow the expression of the polytope protein. Western blotting and indirect immunofluorescence antibody staining of polytope vaccinia infected lymphoblastoid cell lines (LCLs) and the processing defective T2 cell line[6,7] using these antibodies failed to detect polytope protein products (data not shown). Recombinant proteins expressed by vaccinia using the same P7.5 promoter are usually readily detected[24] implying that the polytope protein was rapidly degraded in the cytoplasm of mammalian cells. This degradation was not dependent on LMP2 and 7 since the T2 cell line does not express these proteosome associated endopeptidases[6,7]. This phenomenon is consistent with other studies expressing truncated

proteins or peptides in mammalian cells[25] and is likely to reflect the inability of such proteins to fold into any secondary or tertiary structures.

43. A glutathione S-transferase fusion expression vector containing the human polytope was constructed. The DNA fragment coding for the human polytope was excised from pBSpolytope using BamHI/HincII and cloned into the BamHI/AmaI restriction sites in pGex-3x (GST Gene Fusion System Pharmacia) to make pFuspoly. This plasmid was used to express the polytope fusion in bacteria using the standard induction protocols. An aliquot of the bacteria was lysed in loading buffer and run on a 20% SDS PAGE gel with size markers. This gel indicated that the expected protein of approximately 38kD (the human polytope plus the GST domain (26kD)) was being expressed in bacteria containing the plasmid. Western blotting with the two monoclonal antibodies 8G10/48 and 8E7/55 demonstrated that the fusion detected contained the human polytope which has the two linear B cell epitopes (STNS and NNLVSGPEH respectively) incorporated at each end of the polytope construct. This protein may be incorporated into liposomes or ISCOMs.

44. Attempts to purify the fusion protein using the GST purification employing glutathione agarose beads failed due to the lack of fusion protein in the bacterial extract supernatant. All the fusion protein precipitated with the cell debris. The protocol was not at fault since GST expressed by itself in a different bacterial culture was in the bacterial extract supernatant and could be purified easily. These data suggest the fusion protein is rapidly degraded in the bacteria unless sequestered into bacterial inclusion bodies from which purification using the GST system is difficult.

Example 2

MATERIALS AND METHODS

45. **Construction of a recombinant vaccinia expressing the murine polytope protein.** Ten class I murine CTL epitopes from various diseases were selected so that there were two epitopes for each of H-2Db, H-2Kb, H-2Kd, H-2Kk and H-2Ld which are represented in three strains of mice (see Table 2). These amino acid sequences were arranged such that each of the first 5 epitopes was restricted by a different HLA allele followed by the second group 6-10. The two groups of epitopes were converted to a DNA sequence using the universal codon usage data. These two DNA sequences were separated by an SpeI and flanked by a Xbal restriction

site at the 5' end and a AvrII site at the 3' end. Also incorporated at the 5' end is a BamHI restriction site, a Kozac sequence[13] and a methionine start codon. While at the 3' end there is a B cell epitope from *Plasmodium falciparum*, a stop codon and a SalI restriction site see Figures 4 and 5. Five 75mer oligonucleotides and a 76mer oligonucleotide overlapping by 20 base pairs, representing this 341 base pair sequence, were spliced together using Splicing by Overlap Extension (SOEing)[14] and the polymerase chain reaction (PCR). Primer dimers were made of primers 1 and 2, 3 and 4, 5 and 6 (0.4µg of each) in 40µl reactions containing standard 1x *Pfu* PCR buffer, 0.2 mM dNTPs and 1U of Cloned *Pfu* DNA polymerase (hot start at 94°C) using a Perkin Elmer 9600 PCR machine programmed with the following thermal program; 94°C for 10 seconds, 42°C for 20 seconds and 72°C for 20 seconds for 5 cycles. At the end of 5 cycles the PCR program~~me~~ was paused at 72°C and 20µl aliquots of reactions 2 and 3 were mixed (reaction 1 was left in the PCR machine) and subjected to a further 5 cycles. At cycle 10 the program was paused again and 20µl of reaction 1 added to the combined reactions 2 and 3 and a further 5 cycles completed. The combined 40µl sample was then gel purified on a 4% Nusieve agarose gel (FMC) and a gel slice corresponding to the correct sized fragment removed and spun through Whatmann 3MM paper. Two 20mer oligonucleotides were used to PCR amplify the full length product using the standard reaction mix as above and an annealing temperature of 50°C and 25 cycles. The full length PCR fragment was gel purified in a 4% Nusieve agarose gel, cloned into the EcoRV site of pBluescript IIKS$^-$ to make pBSMP and checked for mutations by sequencing. The DNA insert of a plasmid containing the correct sequence was excised using BamHI/SalI restriction enzymes and cloned, using the same enzymes, behind the vaccinia P7.5 early/late promoter in the plasmid shuttle vector pBCB07[15] to generate pSTMOUSEPOLY. Construction of a TK- recombinant virus was carried out using marker rescue recombination between pSTMOUSEPOLY and VV-WR-L929 using protocols described previously[16]. Plaque purified virus was tested for TK phenotype and for appropriate genome arrangement by Southern blotting of viral DNA[17].

46. **Vaccination of mice with recombinant murine polytope vaccinia.** The recombinant vaccinia was used to vaccinate 3 mice in each of the 3 strains of mice ~~Balb/cv,~~BALB/cv, C57BL/6 and CBA. The vaccinations were I.V. 50µl containing 5 x 10^7 pfu

of vaccinia and the mice were left to recover for three weeks. The TK- vaccinia was used as a negative control for each strain of mouse in this experiment.

47. **Cytotoxic T cell assays.** Splenocytes were harvested from the vaccinated mice 3 weeks post vaccination and restimulated with the appropriate peptides (1μg/ml) in vitro[16]. No peptide were used for restimulations as negative controls. After 7 days of culture the restimulated bulk effectors were harvested and used in a 5 hour, ^{51}Cr-release assays. The targets used in these assays were ConA blasts generated from each of the strains coated with one of the peptides presented by that strain. Three effector to target ratios were used 50:1, 10:1 and 2:1 the results are shown in ~~Figure 6.~~FIGS. 6A-6J.

RESULTS

Construction of murine recombinant polytope vaccinia,

48. The list of epitopes included in the murine polytope are listed in Table 2.

Table 2 CTL epitopes of the murine CTL polytope

~~SOURCE~~	~~SEQUENCE~~	~~RESTRICTION~~	~~MOUSE STRAIN~~
SOURCE	SEQUENCE	SEQ ID NO:	RESTRICTION	MOUSE STRAIN
~~Influenza nuclear protein (366-374)~~	~~ASNENMDAM~~	~~H-2D^b~~	~~C57BL/6~~
Influenza nuclear protein (366-374)	ASNENMDAM	1	H-2D^b	C57BL/6
~~Adenovirus 5 E1A (234-243)~~	~~SGPSNTPPEI~~	~~H-2D^b~~	~~C57BL/6~~
Adenovirus 5 E1A (234-243)	SGPSNTPPEI	14	H-2D^b	C57BL/6
~~Ovalbumin (257-264)~~	~~SIINFEEKL~~	~~H-2K^b~~	~~C57BL/6~~
Ovalbumin (257-264)	SIINFEEKL	15	H-2K^b	C57BL/6
~~Sendai virus nuclear protein (324-332)~~	~~FAPGNYPAL~~	~~H-2K^b~~	~~C57BL/6~~
Sendai virus nuclear protein (324-332)	FAPGNYPAL	5	H-2K^b	C57BL/6
~~Influenza nuclear protein (147-155)~~	~~TYQRTRALV~~	~~H-2K^d~~	~~Balb/c~~
Influenza nuclear protein (147-155)	TYQRTRALV	19	H-2K^d	BALB/c
~~P. Berghei Circumsporozoite protein (249-257)~~	~~SYIPSAEKI~~	~~H-SK^d~~	~~Balb/c~~
P. Berghei Circumsporozoite protein (249-257)	SYIPSAEKI	18	H-SK^d	BALB/c
~~Influenza nuclear protein (50-58)~~	~~SDYEGRLI~~	~~H-2K^k~~	~~CBA~~
Influenza nuclear protein (50-58)	SDYEGRLI	13	H-2K^k	CBA
~~Influenza NS1 (152-160)~~	~~EEGAIVGEI~~	~~H-SK^k~~	~~CBA~~
Influenza NS1 (152-160)	EEGAIVGEI	3	H-SK^k	CBA
~~Murine Cytomegalovirus pp89 (168-176)~~	~~YPHFMPTNL~~	~~H-2L^d~~	~~Balb/c~~
Murine Cytomegalovirus pp89 (168-176)	YPHFMPTNL	20	H-2L^d	BALB/c
~~Lymphocytic choriomeningitis virus nuclear protein (118-126)~~	~~RPQASGVYM~~	~~H-SL^d~~	~~Balb/c~~

Lymphocytic choriomeningitis virus nuclear protein (118-126)	RPQASGVYM	12	H-SL[d]	BALB/c

49. The construction of the polytope DNA insert is ~~summarised in Fig.~~summarized in FIG. 4. The polytope sequence is shown in Fig 5.

CTL assays.

50. Each epitope in the polytope induced a primary CTL response in mice with the appropriately MHC allele. No competition between two epitopes restricted by the same allele was observed. (the high flu NP response in CBA mice given TK- controls is likely to be due to a naturally acquired influenza).

51. Polytope constructs containing multiple CTL epitopes from various pathogens restricted by various MHC alleles are clearly capable of generating primary CTL responses to each epitope within the polytope vaccine. This has clear application in all vaccines where CTL responses are required for protection. For instance, multiple HIV CTL epitopes might be combined in a therapeutic vaccine to foreshadow epitopes expressed by escape mutants and thereby prevent disease progression.

52. Murine polyepitope mice have SIINFEKL specific CTL which can kill the ovalbumin transfected cell line EG7 in vitro and in vivo.

SIINFEKL specific CTL which kill the EG7 tumor cells demonstrated in vitro

53. Spleen cells from murine vaccinia ~~immunised~~immunized mice were collected 4 weeks post vaccination and restimulated in vitro with 10ug/ml SIINFEKL for 7 days. Effectors could not lyse the untransfected parent line EL4 but could lyse the EG7 tumo~~u~~r cells and EL4 cells ~~sensitised~~sensitized with SIINFEKL.

Protection against EG7 tumo~~u~~r in vivo afforded by murine polytope

54. Mice (C57B6) were given either human polytope vaccinia ~~(Thomsom~~(Thomson et al., 1995) or murine polytope vaccinia (10^7 pfu/mouse/ip) and 4 weeks later received 10^7 EL4 or EG7 tumo~~u~~r cells (Moore et al., 1988. Cell 54,777) subcutaneously (10 or 11 mice per group).

55. The number of mice with ~~visable tumours~~visible tumors (all were >1cm diameter) at day 9 is given.

Human Polytope Vaccinia		Murine Polytope Vaccinia	
EG7	EL4	EG7	EL4
10/10*	10/10	0/11	10/10

*(Two mice had tumours <1cm in diameter)

Protection against MCMV

56. BALB/c mice were challenged with MCMV (K181 strain, 8 x 10^3 PFU, 100µl intraperitoneally) 5 weeks after polytope vaccinia vaccination. Four days after challenge the viral titres per gram of spleen were determined the results are shown in ~~Fig.7~~FIG.7 (method of Scalzo *et al*)[17].

Evaluation of polytope vaccines delivered in a DNA plasmid.

57. The polytope protein described above contained a linear antibody epitope ~~recognised~~recognized by a monoclonal antibody. As described above the polytope protein could not, however, be detected in cells infected with the polytope vaccinia indicating that it is very unstable; a likely consequence of having no folding structure. It was thus considered that delivery of a polytope vaccine may be best achieved using nucleic acid vaccination technology or with an adjuvant system that protects from proteolysis ~~(eg liposomes~~(e.g. liposomes or ISCOMs).

~~The CMV promotor~~58. The CMV promoter cassette from pCIS2.CXXNH (Eaton *et al* (1986) *Biochemistry* **25**(26) p8343) was cloned into the EcoRI site of pUC8 in the same orientation as the LacZ gene to make the plasmid pDNAVacc (used as a control plasmid in the DNA vaccination experiments). This plasmid then had the murine polytope (from pBSMP) inserted into the XhoI site in the multiple cloning site to form pSTMPDV. The plasmid pRSVGM/CMVMP has fragments sourced from a number of different plasmids. The RSV ~~promotor~~promoter was excised from pRSVHygro (Long *et al* (1991) *Hum. Immunol.* **31**, 229-235), the murine GM-CSF gene from pMPZen(GM-CSF) (Johnson *et al* (1989) *EMBO* **8**, 441-448) and the CMV ~~promotor~~promoter cassette from pCS (Kienzie *et al* (1992) *Arch. Virol.* **124** p123-132). Into the CMV cassette was the murine polytope cloned into the SmaI site of the multiple cloning site. Both genes , murine GM-CSF and the murine polytope, use the bi-directional polyA from SV40.

59. Nine 6 week old female ~~Balb/c~~BALB/c mice were injected I.M. with 50µg of either pDNAVacc (plasmid control), pSTMPDV (murine polytope only) or pRSVGM/CMVMP (murine GM-CSF and murine polytope) in 50µl of PBS (see next figure). They were given boosters with another 50µg of the same plasmids at 3 weeks. At 8 weeks from the vaccination these mice were killed and their spleens removed. Splenocytes were isolated and cultured with peptide as previously described for vaccinia vaccinated animals. These bulk effectors were then used in standard ^{51}Cr release assays against P815 cells coated with peptide corresponding to the epitopes in the murine polytope that are presented by ~~Balb/c~~BALB/c mice. The assay was done for 6 hours at E:T ratios of 2:1, 10:1 and 50:1.

60. The results of these experiments are shown in Fig 8.

SPECIFIC CTL ACTIVITY AGAINST PEPTIDE COATED AND VIRUS INFECTED TARGETS INDUCED BY THE MURINE POLYTOPE VACCINIA

Method

61. **1. Vaccination and Effector Cell Preparation**. Mice (3 per group) were vaccinated intraperitoneally (IP) with 5 x 10^7 PFU vaccinia. Mice were boosted via the same route and with the same amount of vaccinia week 3. The spleens were removed 6 weeks after the initial vaccination and the splenocytes were isolated after erythrocyte lysis with ACK Buffer (0.15M NH_4Cl, 1mM $KHCO_3$, 0.1mM Na_2EDTA)(Current Protocols in Immunology, Ed JE Coligan, AM Kruisbeek, DH Margulies, EM Shevach, W Strober, 1994 John Wiley and Sons Inc. USA.). 5 x 10^6 splenocytes per well were peptide restimulated (1µg/ml) in bulk T cell media (RPMI/10% Fetal Calf Serum (FCS), 2mM Glutamine, $5x10^{-5}$M 2-Mercaptoethanol) for seven days prior to cytotoxic T lymphocyte (CTL) assay on ^{51}chromium (^{51}Cr) labelled target cells[17]. The peptides used for restimulation are given above A to J. The effectors were used against either peptide coated targets A-J, viral infected targets (A'-J') or transfected antigen expressing targets (I').

62. **2. Preparation of Target Cells.** Cell lines used as targets in these assays were P815 for ~~Balb/c~~BALB/c (H-2^d), EL-4 and EG7 for C57BL/6 (H-2^b), L929 for CBA (H-2^k) L929, or con A blasts prepared from the ~~Balb/c,~~BALB/c, C57BL/6 or CBA mice, respectively.[1] To express the required epitope for CTL killing, target cells were either pre-incubated with (i) peptide (A-J), (ii) vaccinia (B'-D', F'-J'). or (iii) Influenza (A', E'), or maintained as the (iv)

Ovalbumin-expressing plasmid transfectant of El-4 (EG7) in the case of the SIINFEKL epitope system (I').

63. (i) Peptide coated targets (A-J): Target cells were centrifuged at 1000rpm/5 min. The supernatant was discarded to approximately 200µg/ml and 10-20µl of either RPMI (No peptide) or 200ug/ml stock peptide in RPMI (peptide coated) (final concentration 10µg/ml) was added to the cell pellet. One hundred ~~microlitres~~microliters of ^{51}Cr was added to cell pellet and the cells were incubated at 37°C for 1 hr. The cells were then washed twice with RPMI/10%FCS through a FCS underlayer and resuspended at 10^5/ml for target cells in the CTL assay.

64. (ii) Vaccinia (Vacc.) infected targets (B'-D', F'-J'): Vaccinia used for virus infected targets were the Murine Polytope (Vacc Mu PT), with the Human Polytope (Vacc Hu PT) as the negative control. Cell lines infected by vaccinia were P815 (B'-D'), L929 (F') and EL-4 (G'-J'). The target cells were centrifuged at 1000rpm/5 min. The supernatant was discarded to approximately 200ul and the cells (approx. 10^6 cells) infected with vaccinia at a multiplicity of infection (MOI) of 10:1 by adding 20µl vaccinia (10^9 pfu/ml) followed by incubation for 1 hr at 37°C. Five ~~millilitres~~milliliters of RPMI/10%FCS was then added, cells mixed and incubated overnight at 37°C. These cells were subsequently centrifuged and supernatant discarded into camdyne. One hundred ~~microlitres~~microliters of ^{51}Cr was added to cell pellet and the cells incubated at 37°C for 1 hr. The cells were then washed twice with RPMI/10%FCS through a FCS underlayer and resuspended at 10^5/ml for target cells in the CTL assay.

65. (iii) Influenza infected targets (A', E'): The A/PR/8/34 strain of Influenza virus was used for the ~~Balb/c~~BALB/c targets (A') and the reassortant A/Taiwan/1/86 (IVR-40) for the CBA targets (E'). Allantoic fluid was used as the negative control. Cell lines infected by influenza were P815 (A') and L929 (E'). Target cells were centrifuged at 1000rpm/5 min. and supernatant was discarded. Five hundred ~~microlitres:~~microliters: 50µl Influenza virus (10^8/ml EID) or Allantoic Fluid, 50µl ^{51}Cr, 400µl RPMI/1%FCS was added to the cell pellet and incubated for 1 hr at 37°C. Ten ~~millilitres~~milliliters of RPMI/10%FCS was added, mixed and incubated a further 2 hr at 37°C. The cells were then washed twice with RPMI/10%FCS through a FCS underlayer and resuspended at 10^5/ml for target cells in CTL assay.

66. (iv) Ovalbumin expressing targets (I'): EG7 cells are EL-4 cells transfected with an expression plasmid containing chicken ovalbumin cDNA (Moore MW, Carbone FR and Bevan BJ (1988) Introduction of soluble protein into Class 1 pathway of antigen processing and presentation. *Cell* 54: 777-785.). These cells were maintained in RPMI/10% FCS. 20mM Hepes, 2mM Glutamine, 1mM Na Pyruvate, 1001U/ml penicillin and 100µg/ml Streptomycin. The plasmid was selected and maintained in Geneticin (G-418) at 500µg/ml once per month. EL-4 cells with no peptide (EL4 no pep) were used as the negative control. The cells were centrifuged at 1000rpm/5 min. and supernatant discarded to approximately 200µl. One hundred ~~microlitres~~microliters of ^{51}Cr was added to cell pellet and the cells incubated at 37°C for 1 hr. The cells were then washed twice with RPMI/10%FCS through a FCS underlayer and resuspended at 10^5/ml for target cells in the CTL assay.

67. **3. CTL Assay.** The restimulated splenocytes ($5x10^6$/ml) were dispensed (100µl) in triplicate at three Effector: Target ratios (50, 10, $2x10^4$ effector cells: $1x10^4$ target cells for the CTL assay. One hundred ~~microlitres~~microliters of target cells (10^5/ml) were added to all effectors and control wells (Spontaneous release = 100µl media; Maximal release = 100µl 0.5% SDS/ RPMI/10%FCS). Microtitre plates were centrifuged at 500rpm for 5 min. and incubated at 37°C for 6hr. Plates were recentrifuged at 500rpm/5 min. and 25µl of supernatant was counted for ^{51}Cr release. Percentage Specific Lysis represents averages of triplicate counts: 100 x (Test cpm - Spontaneous cpm)/(Maximal cpm - Spontaneous cpm).

68. The results are shown in ~~Figure 9.~~FIGS. 9-28.

DNA vaccination experiment

69. The initial DNA vaccination experiments illustrate that the polytope can be delivered using DNA vaccination. In addition, that vaccination may be improved by the co-delivery of a cytokine gene (GM-CSF), although in this experiment the improvement is not statistically significant.

70. The current system is clearly sub-optimal. Likely improvements would be the use of potentially better plasmid vectors e.g. the vectors from Vical, San Diego (Sedegah M, R Hedstrom, P Hobart, SL Hoffman, 1994. Protection against malaria by ~~immunisation~~immunization with plasmid DNA encoding circumsporozoite protein. PNAS 91, 9866-9870) and the use of better delivery systems (to IM injection) employing a gene gun (Sun

WH., Burkholder JK., Sun J., Culp J., Lu XG., Pugh TD., Ershler WB, Yang NS. IN VIVO CYTOKINE GENE TRANSFER BY GENE GUN REDUCES TUMOUR GROWTH IN MICE. Proceedings of the National Academy of Sciences of the United States of America. 92:2889-2893, 1995.). In addition priming against CTL epitopes usually requires CD4 T cell help[17] thus the inclusion helper epitopes or proteins in the construct may improve the level and reliability of CTL priming by the murine DNA vaccine polytope.

71. **Lack of "Original antigenic sin" or the ability of a polytope to raise immune responses to all the epitopes in a polytope when the individual has already got a response to one of the epitopes.**

Introduction

72. Original antigenic sin is a term given to an antibody based phenomena whereby an existing antibody response to an epitope prevents the raising of an immune response to a second epitope when that epitope is present on the same protein as the first epitope (Benjamini E., Andria M.L., Estin C.D., Notron, F.L. & Leung C.Y. (1988) Studies on the clonality of the response to an epitope of a protein antigen. Randomness of activation of epitope -recognizing clones and the development of clonal dominance. *J. Immunol.* 141,55.). The reason for this phenomena is that large population of primed B cells specific for the first epitope bind and mop up all the available antigen before a naive B cell specific for the second antibody has a chance to bind the antigen, process it and present it to T helper cells. A similar situation might occur when an individual is vaccinated with a polytope when he/she already has a response to one of the epitopes in the polytope. The existing CTL might kill all the polytope expressing cells before any of the other epitopes can be presented to naive T cells.

Method

73. To test this possibility mice ~~(Balb/c)~~(BALB/c) were infected with 10^4 pfu of Murine cytomegalovirus (MCMV) (K181 strain - Scalzo et al. 1995) and left for 5 weeks at which point strong CTL responses specific for the MCMV epitope, YPHFMPTNL, had developed (Scalzo et al. 1995 - Fig 2A). These mice were then given the murine polytope vaccinia and spleen cells assayed 10 days later for CTL specific for the three other epitopes presented by the polytope in this strain of mouse (RPQASGVYM, Lymphocytic

choriomeningitis virus nuclear protein, H-2L^d; TYQRTRALV, influenza nuclear protein, H-2K^d and SYIPSAEKI, P. Berghei circumsporozoite protein, H-2K^d).

Results

74. Responses to each of the three new epitopes was observed following polytope vaccination, illustrating that the YPHFMPTNL specific CTL did not prevent priming of CTL specific for RPQASGVYM, TYQRTRALV and SYIPSAEKI when all four epitopes are presented together in the polytope. (Control animals receiving the human polytope vaccinia instead of the murine polytope vaccinia, showed only YPHFMPTNL specific CTL).

75. This series of experiments illustrate that if a polytope was, for instance, designed to cover a variety of different diseases, an individual receiving such polytope vaccine, but who had already been exposed to one of the target diseases would still be ~~immunised~~immunized against the remaining CTL epitopes in the polytope.

76. As will be apparent to those skilled in the art the present inventors have shown that the natural flanking sequences of CTL epitopes are not required for class I processing, that is each epitope within the polyepitope protein was always efficiently processed and presented to appropriate CTL clones by autologous polyepitope vaccinia infected target cells. It will be apparent to those skilled in the art that the polytopes may include sequences not naturally found to flank the epitopes.

~~As discussed~~77. As discussed above the present invention can be used with a range of epitopes. A range of epitopes are now available on an Internet address which is described in Brusic *et al* Nucleic Acids Research, 1994, 22; 3663-5.

78. It will be appreciated by persons skilled in the art that numerous variations and/or modifications may be made to the invention as shown in the specific embodiments without departing from the spirit or scope of the invention as broadly described. The present embodiments are, therefore, to be considered in all respects as illustrative and not restrictive.

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ABSTRACT

The present invention relates to a recombinant polyepitope cytotoxic T lymphocyte vaccine. The vaccine comprises at least one recombinant protein including a plurality of cytotoxic T lymphocyte epitopes from one or more pathogens, wherein the at least one recombinant protein is substantially free of sequences naturally found to flank the cytotoxic T lymphocyte epitopes. In addition the present invention also provides a polynucleotide including at least one sequence encoding a plurality of cytotoxic T lymphocyte epitopes from one or more pathogens.

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A pattern search method for putative anchor residues in T cell epitopes

The binding affinity between an antigenic peptide and its particular major histocompatibility complex (MHC) molecule seems to be largely determined by only a few residues. These residues have been called "anchors" because of their property of fitting into "pockets" inside the groove of the MHC molecule. To predict natural antigenic epitopes within a longer sequence, it therefore appears to be important to know the motif or pattern describing the anchors, *i.e.* the anchors amino acid residue preference and the distance between anchor residues.

A large set of MHC class I-restricted peptides has been described. Peptide sequences vary in length and lack an obvious common sequence motif. For a list of peptides belonging to one type of MHC class I molecule, we describe a method to find the most prominent sequence motif with at least two anchor residues. Briefly, antigenic sequences are aligned, and two anchor positions are searched for, where all anchor residues share a high similarity. The alignments are scored according to the similarity of their anchor residues. We show that the motifs predicted for the MHC alleles A2.1, B27, K^b, K^d, D^b are in substantial agreement with experimental data. We derive binding motifs for the MHC class I alleles HLA-A1, A11, B8, B14, H-2L^d and for the MHC class II alleles I-A^b and I-A^s. In some cases, higher scores were obtained by allowing a slight variation in the number of residues between anchors. Therefore, we support the view that the length of epitopes belonging to a particular class I MHC is not uniform.

This method can be used to predict the natural short epitope inside longer antigenic peptides and to predict the epitopes anchor residues. Anchor motifs can be used to search for antigenic regions in sequences of infectious viruses, bacteria and parasites.

1 Introduction

CD8-positive T lymphocytes are an important component in host defense against viral infection. They can recognize small virus-derived protein fragments presented by the MHC class I proteins at the cell surface of infected cells. Recognition is mediated by the T cell receptor (TCR)-CD8 complex and results in the lysis of infected cells. CTL can be protective against virus infection by adaptive transfer of specific CTL [1]. Also immunization with MHC class I-restricted peptides has been successful [2]. Thus, defining minimal antigenic peptides is essential for the design of highly specific vaccines based on the induction of cellular immunity.

Which are the common features of peptides presented by MHC class I proteins? Initial attempts to address this question were aimed at finding properties common to all peptides, irrespective of the particular restricting element. DeLisi et al. [3–5] found a pattern consistent with an amphipathic helix having hydrophilic residues on one face and hydrophobic residues on the opposite face. Rothbard and Taylor came to a similar conclusion [6], namely, that class I antigenic peptides share a pattern with few hydrophobic boxes and helical formation. Nuclear magnetic resonance studies on immunogenic peptides from the HIV envelope glycoprotein gp120 were consistent with a turn and/or helix formation in water [7]. A modelling study from Rognan et al. [8] predicts an α-helical conformation of a peptide bound to H-2-K^d. In contrast, Sette and coworkers proposed a planar conformation for peptides bound to class II molecules [9, 10]. Indeed, the recent structure determinations of HLA-B27-peptide and H-2-K^d-peptide complexes rebuffed the helical model, but showed a largely extended conformation of the peptide inside the binding groove [11–14].

A standard strategy to find MHC class I-restricted peptide motifs used to be the mapping of antigenic regions with synthetic peptides and their subsequent truncation and/or the substitution of individual amino acid residues [15–29]. In this work, we try to use antigenic peptide sequences to derive a sequence pattern specific for a particular class I or class II molecule. Such motifs may be useful for the prediction of antigenic sites in viral proteins, they may reduce the number of peptides that have to be screened in lysis assays, and the motifs may facilitate the development of synthetic peptide vaccines with multiple MHC-type specificity [2, 30–36].

We used some recently discovered motifs to test the validity of our predictions. Several groups have successfully isolated peptides directly from cellular MHC proteins [37–40]. Subsequent sequencing of the eluted peptide mixture showed that some positions are dominated by one or a few amino acid residues with similar physico-chemical proper-

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ties, while other positions appeared to be highly variable. The motifs deduced were MHC-type dependent. It is tempting to speculate that the invariant positions coincide with the binding positions, where multiple interactions between peptide side chains and the pocket-forming HLA residues results in the strong binding affinity. Indeed, this has been shown to be true in the case of HLA-B27 and H2-K^b [11, 12]. Together, these data underlined the widely accepted assumption that, for class I molecules, a few positions in the sequence are crucial for binding and the length of the presented peptide is uniform [21, 37, 41–44]. Our findings support the recent view that the length of MHC class I presented epitopes is not strictly constant [11, 44].

2 Materials and methods

2.1 Collection of epitope sequences

Scanning the scientific literature, we collected sequences of peptides which are restricted by MHC class I molecules and elicit a cytotoxic T cell response, or, in the case of MHC class II epitopes, were eluted from the respective HLA molecule and sequenced subsequently. Only those MHC types are considered where at least three different peptides are known (see Table 3).

2.2 Algorithm to find a common sequence motif

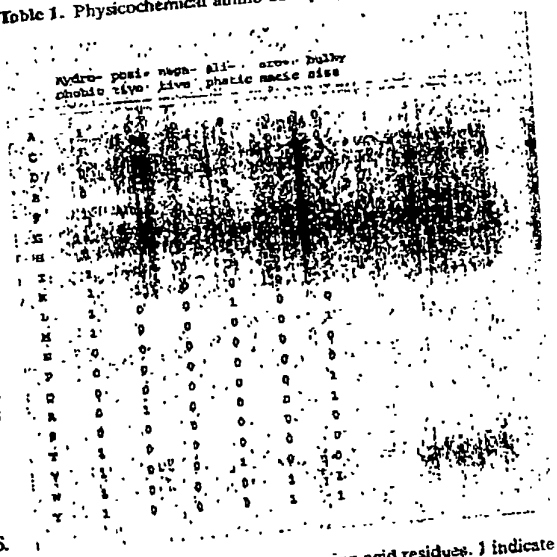
A program FIND-MOTIF was written in C under SUN-OS. No hardware specific functions were used, so it can be implemented easily on any computer. Briefly, peptide sequences antigenic in combination with a particular MHC class I protein are aligned over a window of length 6. A window was chosen to reduce the number of alignments by avoiding those alignments with a too-narrow overlap of sequences. All possible alignments that had one position of high similarity were checked. The similarity at a particular window position was calculated by adding similarity values of all possible residue pairs. If this overall similarity value (after normalization, see Sect. 2.4) was higher than the mean of highest and lowest value in the similarity matrix, the alignment was stored for further processing.

In the next step, an all-against-all comparison of one-anchor alignments was performed. Alignments in which the distance between two anchors varies by not more then plus or minus one residue for all sequences were merged and collected. To speed up the program, a minimal distance of two residues between two anchors was fixed. This seemed plausible since the known motifs show at least two residues between anchors.

2.3 Amino acid similarity matrices

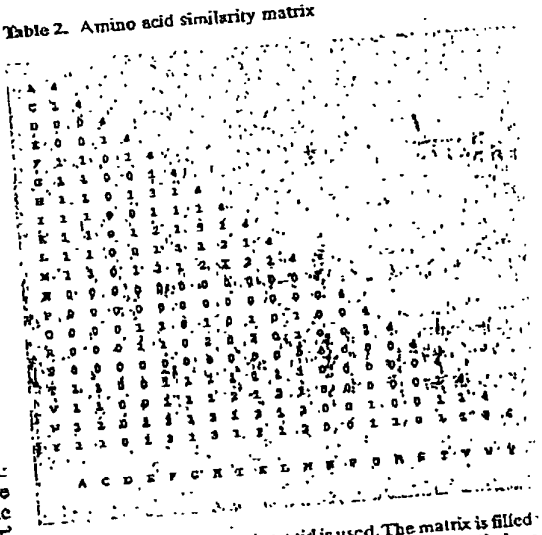
Four different amino acid similarity matrices were tested: (a) the Dayhoff matrix PAM250 [45] as implemented in the GCG protein analysis package [46]; (b) the three inside matrices 'Inside Alpha', 'Inside Beta' and 'Inside Other' from Luethy et al. [47] were merged by us to one inside matrix; (c) the substitution probability table for inaccessible residues from Overington et al. [48]; (d) a similarity matrix (Table 2) derived from a table with simple physico-chemical amino acid properties (Table 1) [49]. For each pair of residues, the physico chemical properties in common were counted from Table 1 and stored in Table 2.

Table 1. Physicochemical amino acid properties



The one letter code was used for amino acid residues. 1 indicate that an amino acid residue has the particular property.

Table 2. Amino acid similarity matrix



The one letter code for amino acid is used. The matrix is filled the number of physico-chemical properties (see Table 1) share a pair of residues. The diagonal is set by hand.

The diagonal was set by hand to the maximal value found in the respective matrix.

2.4 Score

To rank the alignments, a score was calculated for each alignment. For both anchor positions, an all-against-all residue comparison was performed, and similarity values from Table 2 were added up. The sum was divided by the number of comparisons to compare the score coming from different epitope lists.

2.5 Selection of the best alignment

For one list of peptide sequences, we show the alignment with highest score (see Table 3). If more than one alignment results in the same score, we show the alignment with the shortest overall gap length.

3 Results and discussion

The program FIND-MOTIF provides a tool to align a set of peptide sequences according to the highest similarity at a given number of positions. This cannot be done easily "by eye" since the number of possible alignments is high even in the case of a few short sequences. For instance, seven peptide sequences belonging to HLA-B8 and varying in length from 8 to 25 residues (Table 3) can be aligned in 7603200 ways over a window of length 6.

In this work we applied the method to predict anchor residues for T cell epitopes restricted by different MHC class I and class II proteins. Other available programs for multiple sequence alignments like CLUSTAL [50], MAXHOM [51] or GCG-PILEUP [46] are not applicable for such a study because different gap penalties for inside and end gaps cannot be applied, and it is not possible to maximize the alignment score by counting only a few positions while neglecting all other positions.

Alignments were ranked according to the degree of similarity in the two most conserved positions. We focused on two anchors, because this seems to be the minimal number required to attach a peptide to the MHC protein. Van Bleek and Nathenson found a major motif of tyrosine and phenylalanine for peptides binding to K^b [52]; Jardetzky et al. propose that three to four amino acid side chains point down into the HLA-B27 groove [39]. All motifs for A2, D^b, K^b and K^d from Falk et al. have two "dominant anchor" positions [38]. For a class II protein, Kropshofer et al. deduced a two-residue contact model for DR-1-restricted antigens from circular dichroism experiments [53].

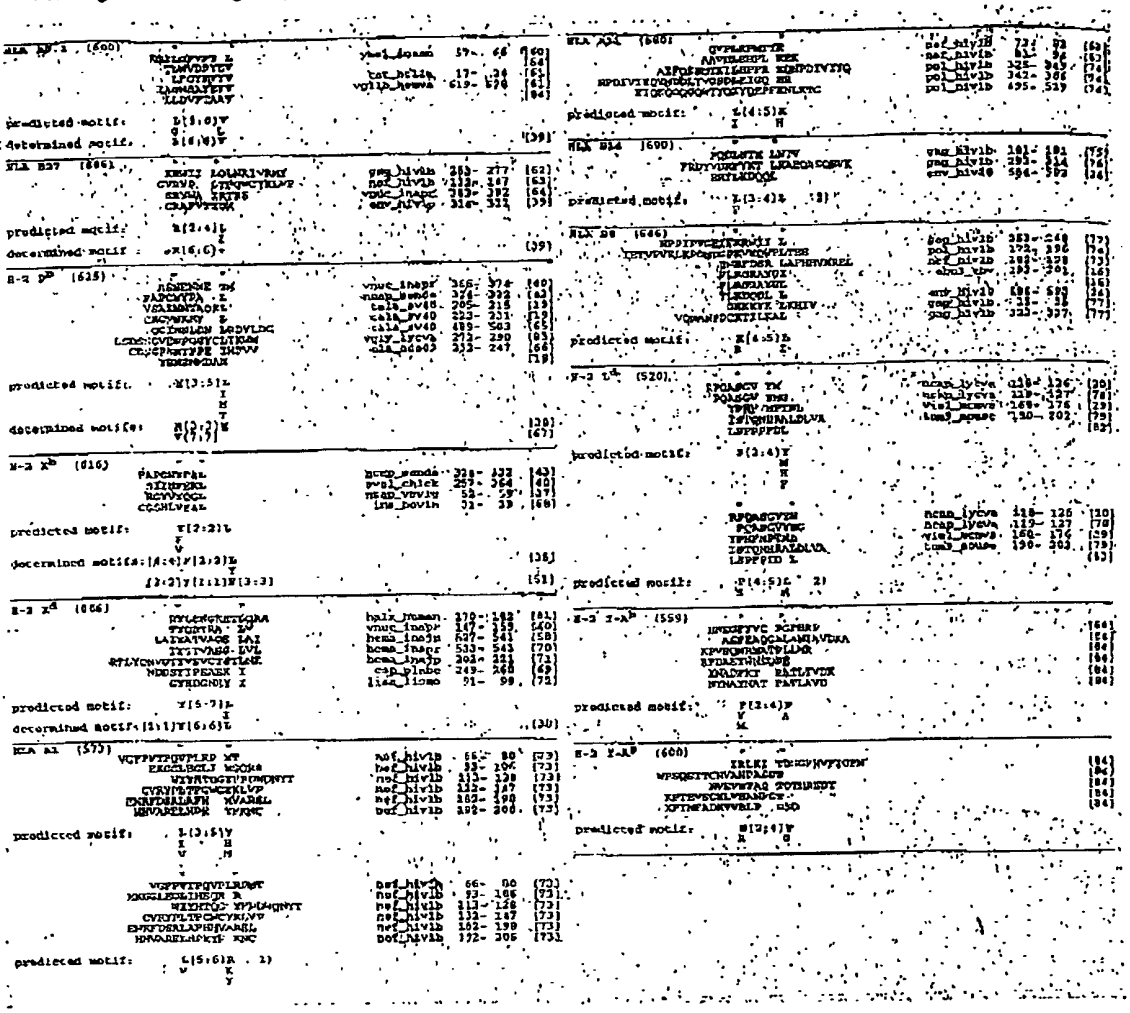
Sequence alignments depend sensibly on the amino acid similarity matrix. We tested the widely used Dayhoff-matrix [45] as well as two matrices derived for buried, inaccessible residues [47, 48]. The interaction between peptide and MHC molecule is certainly better represented by the latter. Unfortunately, initial runs with these published matrices did not lead to the known motif in all cases (data not shown). Finally, we used a simple exchange matrix, derived by counting some physico-chemical properties (Table 1) that are shared by two amino acid residues (Table 2) [49]. This matrix performed surprisingly well. We found the best overall correspondence to the test cases. The anchor positions indicated by the program are in agreement with the known motifs for D^b, K^b, K^d and A2.1 and in partial agreement with the motif published for B27 (Table 3). In the case of HLA-A1 and L^d, however, we show also the alignment using the similarity matrix of Overington et al. [48]. Both alignments have relatively low scores, and these were the only cases, where an alignment using a similarity matrix other than the simple property matrix showed higher conservation for anchor positions.

In preliminary runs we used a fixed distance between two anchor positions. However, in some cases high scoring alignments were obtained only with an unacceptable low similarity threshold, although most peptides shared anchors with high similarity, except few outliers. The outlier peptides, however, had residues of high similarity just next to the (wrong) anchor (data not shown). Stimulated by these observations, we relaxed the distance criterion and allowed the distance between anchors to differ by plus or minus one residue, *i.e.* the distance between anchors may differ by two residues. Provided that such acceptance of a gap in the alignment is realistic, this would have important implications: (i) the distance between anchors, counted as number of amino acid residues, may be slightly different; (ii) the length of peptides presented by a particular MHC molecule may be not unique, but the MHC groove could accommodate peptides of slightly different length. Indeed, very recent experimental data show some length variation of peptides bound to H-2 K^b [44], and (iii) the original conformation of the peptide may be of minor importance for binding. The modeling study of Rognan et al. [8] and the work of Rini et al. [54] are in line with such an "induced fit", as are the findings of Maryanski et al.: they substituted six residues between two K^d anchors by five prolines without disturbing the T cell response. However, the choice of residues between anchors may not be completely random because another substitution against five glycines nearly disrupted the T cell response [25].

We applied the method to other peptides restricted by the MHC class I types A1, A11, B8, B14, L^d and peptides restricted by the MHC class II types I-A^s and I-A^b and derived sequence motifs with putative anchors (Table 3).

How realistic are the motifs? The X-ray structure of an HLA-B27/peptide complex demonstrates that the main contact points are in the B- and F-pocket inside the groove. A positively charged arginine of the peptide contacts the negative charged glutamic acid in the B-pocket, and another charged residue points into the F-pocket [14]. The putative charged anchors for B8 and A11 can be rationalized in a similar way. Using alignments of HLA sequences, one can deduce the residues forming the pockets. Provided that restricted peptides have the same orientation inside the groove as in HLA-B27 (which is still a matter of discussion [55–57]) the putative lysine anchor of HLA-A11-restricted peptides might interact with two negatively charged aspartic acids at position 77 and 116 in the F-pocket of HLA-A11. The putative lysine anchor of HLA-B8-restricted peptides might interact with two negatively charged residues in the B-pocket of the HLA-B8 haplotype HLA-B0801 (aspartic acid 9 and glutamic acid

Table 3. Alignments of antigenic peptide sequences



a) Sequences are aligned in groups according to their MHC restricting element and written in one letter code for amino acid residues. Putative anchor residues are indicated by bold letters and an asterisk.

b) The identifier in column 2 and the sequence positions in column 3,4 refer to the Swiss-Prot database [80]. Position data are result of a data base search and may deviate from those given in the original literature (right column: references).

c) The alignment score is given behind the MHC identifier. Only the top scoring alignment (see 2.5) is shown.

d) Gaps in alignments should be read as evenly distributed between anchor positions and are not belonging to a particular position.

e) The motif syntax is explained by example:
[1 : 4] one to four amino acid residues
[2 : 2] two amino acid residues
+ positive amino acid residues
"Dominating" anchor residues are in bold letters.

1) For HLA-A1 and H-2I,^{d} also the alignment using the amino acid similarity matrix from Overington et al. [48] is shown (see Sect. 3).

2) In the case of HLA-B14 several different alignments with the same score were calculated. We show the alignment, which coincides with the alignment calculated using the matrix from Overington et al.

45). Similar deductions for non-charged residues, however, are very speculative in the absence of knowledge about the HLA-molecule structure. In a simplistic way it would seem as if the MHC proteins are built up of specific binding modules in different combinations: HLA-A2.1 may have two hydrophobic pockets, B8 may have a negative and a hydrophobic pocket, A11 may have a hydrophobic and a negative pocket, and B27 may have two positive pockets. In this respect recombinant MHC proteins should give rise to recombined peptide binding specificity.

How reliable is the method? We used random sequences to test the reliability of the method by exchanging one or more peptide sequences in a list by sequences of the same length, but with random amino acid composition. It appeared that about 60–80 % of all peptide sequences in the list must have a similar anchor to find the correct motif, depending on the similarity of anchor positions. In other words, the algorithm tolerates a subset of "wrong" epitopes. Those random sequences could sometimes be identified when several alignments had the same score. Random sequences showed up by changing their position in different alignments, while all real sequences kept place (data not shown). Other validations are the absolute alignment score, which can be compared between different MHC subtypes. Also, the score difference between first and second best scoring alignment can give an indication or reliability: the higher this difference, the more "reliable" the best alignment.

Peptides of a particular group may belong to different MHC subtypes. Such haplotypes are not differentiated by the normal serological typing. Since the polymorphism of the MHC proteins lies mainly in the binding region, haplotypes may have completely different pocket shapes and binding properties. However, the problem of different haplotypes is not as severe in highly inbred strains of mice. This may also be reflected in the good agreement between predicted and known motifs for the mouse subtypes K^d, K^b, D^b and the partial agreement between predicted and known motifs for the human B27 MHC subtype. For instance, two of the five HLA-A2.1 peptide sequences in Table 3 do not have the XLXXXXXXV motif [38], two of the four HLA-B27 peptide sequences do not have the +RXXXXXX+ motif (+ being a positively charged residue, X a residue of any type) [14, 39]. Those peptides might belong to a different haplotype. Another problem may be that peptides identified by CTL stimulation assays are present in large excess during the assay. These non-physiological conditions may indicate greater MHC tolerance for peptide length and sequence variation than is actually the case. In conclusion, the method can give reliable results only if largely homogeneous data are used.

4 Concluding remarks

The computer program FIND-MOTIF can be a valuable tool to derive characteristic motifs from sets of antigenic peptide sequences. Such motifs can be used to search for potentially antigenic sites in sequences of infectious viruses in highly conserved regions. Knowing the HLA haplotypes of an infected individual, peptidic vaccines could be tailored according to the respective infection and HLA haplotyping, *i.e.* peptides containing several epitopes could be synthesized for administration. Since the concentration of antigen may be a critically determining factor for the stimulation of a T cell response [58, 59], even those peptides which do not stimulate a T cell response after an infection might work as a vaccine when applied in excess.

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ANNIVERSARY REVIEW

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MHC ligands and peptide motifs: first listing

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Introduction

The purpose of this article is to provide a compendium of major histocompatibility complex (MHC) peptide motifs and MHC ligands known to date, together with a discussion of the methods used to gain this information. A problem here is the exponential growth of information in this field over the recent years. The number of known MHC ligands was zero in 1989 and three in 1990. This article, written in 1994, lists a couple of hundred such ligands, plus a large number of likely ligands. By the time this work is published, we expect a lot more ligands to be known. On the other hand, the peptide motifs of many of the more important MHC class I molecules are known already, so that this article will still be useful as a source of information. For class II, the situation is a bit different. Only a few allele-specific motifs have been reported, and the data from different authors are partially conflicting. The principles of allele-specific ligand motifs, however, have emerged recently by the combination of information on MHC class II structure, ligand sequencing, and peptide binding assays. Thus, these principles can be applied to further ligands to be identified.

Overview of MHC function

MHC molecules are peptide receptors. Their function is to collect peptides inside the cell and to transport them to the cell surface, where the complex of peptide and MHC molecule may be recognized by the T-cell receptor (TCR) for antigen of T lymphocytes (Rammensee et al. 1993). In normal cells, MHC-associated peptides are derived from normal, that is, self proteins. During their differentiation, T cells become tolerant to complexes of self peptides and self MHC molecules (Von Boehmer 1992). Thus, if any new peptides, e.g., derived from an infectious agent, occur later, they can be recognized by T cells. Since the specific immune system is regulated by T cells, the trimolecular complex of TCR, MHC molecule, and peptide can be considered a control switch for the immune system. Thus, a study of the molecular interactions between the three parts is essential for our understanding of the immune system.

Two classes of MHC molecules are distinguished, class I and class II. Class I molecules consist of a membrane-inserted heavy chain of about 45000 M_r and a non-covalently attached light chain of 12000 M_r (Klein 1986). The latter is also known as β_2-microglobulin (β_2m). The structure of class I molecules has been resolved by X-ray crystallography (Stern and Wiley 1994). It has some resemblance to a moose's head, whereby the antlers would form a groove that is recognized as a peptide-binding device. HLA-A, B, and C are the "classical" class I molecules of humans, and H-2K, H-2D, and H-2L those of the mouse. Class II molecules are heterodimers consisting of two chains α and β, of similar size (about 30000 M_r), both of which are membrane inserted. HLA-DR, DQ, and DP are the human class II molecules, H-2A and E those of the mouse. Their structure is surprisingly similar to that of class I molecules (Stern and Wiley 1994; Stern et al. 1994; Brown et al. 1993).

All HLA molecules, including the numerous "non-classical", are encoded on chromosome 6, with the exception of β_2m which is on chromosome 15. *H2* genes are on chromosome 17 of the mouse, and the mouse β_2m gene is on chromosome 2.

A peculiarity of MHC genes is their extensive polymorphism, characterized by the presence of dozens or hundreds of alleles in a species. *H2* alleles are designated $H2K^b$, $H2K^d$, $H2K^k$ and so on for class I, and $H2A\alpha^b$, $H2A\alpha^k$, $H2Ab^k$, $H2Eb^d$ and so on for class II, whereby different alleles may differ in as many as 40 amino acids (Klein 1986). The present nomenclature (Bodmer et al. 1994) of *HLA* genes and products (which has been changed several times) is outlined as follows: class I heavy chain

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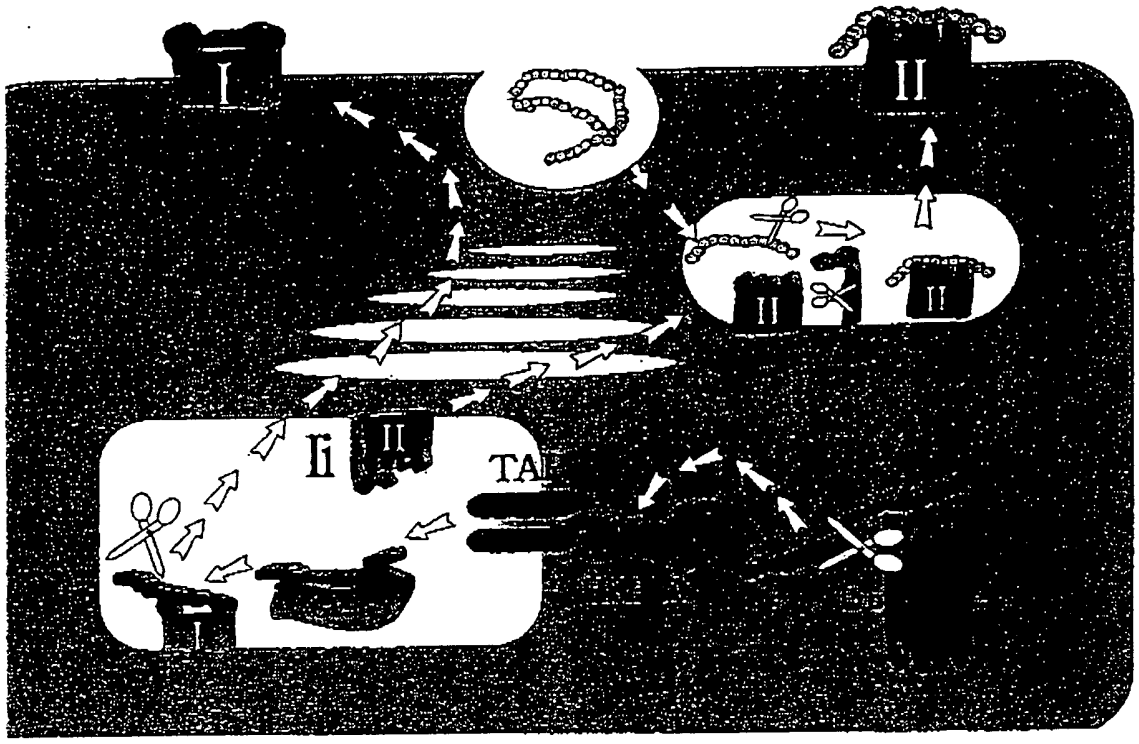

Fig. 1 A simplified and partially hypothetical overview of antigen processing. For explanation see text

loci: *HLA-A, B,* and *C*; class II α chain loci: e.g., *HLA-DRA, DQA1, DPA1*, class II β chain loci: e.g., *HLA-DRB1, DRB3, DQB1, DPB1*. Alleles are designated, for example, *HLA-A*0201*, or *HLA-DRB1*0101*. This nomenclature can only be applied if the respective sequences are known. Since this is not the case in many situations, the old designations, e.g., *HLA-A2* or *HLA-DR3*, based on serology, are still being used, and describe collections of alleles with shared serologic determinants (e.g., *HLA-A2* for *A*0201* through *A*02012*). Both class I light chains and HLA-DRα chains are not very polymorphic (Klein 1986). The high (*HLA-B*) or at least moderate polymorphism of the other genes results in the expression of a large number of combinations of alleles at the different loci per chromosome (haplotype), and in a high degree of heterozygosity. Thus each individual has his or her particular combination of HLA molecules, namely up to six different class I and about six different class II molecules (if the non-classical HLA molecules, whose function is not known, are not considered), making it unlikely to find two unrelated individuals with exactly the same combination of *HLA* genes.

A simplified outline of MHC function is given in the diagram in Figure 1. Class I molecules, both heavy and light chains, are synthesized into the ER (reviewed in Jackson and Peterson 1993). The peptides to be loaded on class I molecules are, in many cases, derived from cytosolic proteins. The details of peptide generation are not known definitely. A widely held view, however, is that cytosolic proteins are partially degraded by an endopeptidase activity of the proteasome, a multisubunit structure with several activities located in the cytosol (Rock et al. 1994). It is not clear, however, how the products of such endopeptidase activity are related to the final class I ligands (Dick et al. 1994). One possibility is that the proteasomes directly produce the correct ligands. Alternatively, proteasomes could cut out larger peptides requiring further processing. The endopeptidase specificity of the proteasome is such that a protein is cut after hydrophobic or charged residues, in principle. The fine specificity of endopeptidase activity is influenced by two proteasome subunits, LMP2 and LMP7, which are encoded in the MHC region and regulated by IFN. However, the exact kind of LMP influence on specificity is controversial (Howard and Seelig 1993). In any case, such peptides must be transported into the ER lumen by the TAP molecule [(transporter associated with processing) (Neefjes and Momburg 1993)]. According to one hypothesis, these peptides are bound and protected from complete degradation by a chaperone, HSP70, before reaching TAP (Srivastava et al. 1994). Peptide transport by TAP molecules has

been directly demonstrated recently (reviewed in Momburg et al. 1994). Transport has specificity especially regarding the C-termini of peptides, and selectivity for peptide lengths. Peptides of 7 to 23 amino acids have been shown to be transported, whereby optima of 10 to 15 amino acids are seen. Human TAPs do not discriminate much between the C-termini of peptides. In contrast, the mouse TAP has a preference for peptides with hydrophobic C-termini and dislikes peptides with charged termini. This pattern of specificities fits well with the peptide specificities of human and mouse MHC class I molecules, since all mouse class I molecules require peptides with hydrophobic C-termini, whereas some human class I molecules require peptides with basic C-termini. On the other hand, mouse cells transfected with the *HLA-A3* gene, requiring peptide ligands with basic C-termini, can be loaded with the fitting peptides (Maier et al. 1994). This indicates that MHC peptide specificity need not be strictly dependent on TAP specificity. That TAP specificity indeed can influence MHC peptide loading is evident from two different TAP forms in the rat, TAP^a and TAP^b. Dependent on co-expressions of the respective TAP, the peptide spectrum of rat MHC class I molecules, $RT1^a$, is different, as indicated by different HPLC behavior of $RT1^a$-associated peptides. When measured in a peptide transporter assay, TAP^a has the same specificity as human TAP, that is, it does not discriminate between hydrophobic and basic C-termini, whereas TAP^b is more like the mouse transporter, with a preference for peptides with hydrophobic C-termini.

Once they are inside the ER lumen, the further fate of transported peptides is not exactly known. The recently reported physical association of TAP molecules and class I molecules suggested that peptides are directly loaded onto class I molecules immediately after discharge from the transporter (Ortmann et al. 1994; Suh et al. 1994). However, this would require that either the incoming peptides are already of the right size for loading to class I molecules, or that they bind as longer peptides (Falk et al. 1990) and are trimmed while somehow bound to MHC. An alternative hypothesis is that peptides are first bound by a chaperone, gp96, which shuttles the peptides to class I molecules, perhaps with some trimming of peptides underway. The main reason for assuming that gp96 plays a role in antigen processing stems from an impressive series of experiments by Srivastava and co-workers (1994), showing that gp96 molecules are associated with a large array of peptides and are able to immunize mice against antigens presented by MHC class I molecules.

In any event, the peptide somehow reaches the class I molecule and binds into the groove, perhaps after a final trimming step while already in touch with MHC. Unusually long peptides found associated with MHC class I molecules might have escaped such a final trimming (Urban et al. 1994). The assembly sequence of class I heavy chain, β_2m and peptide is not quite clear. A recent report indicates that another chaperone, calnexin, is bound to assembled complexes of heavy chain and β_2m, and thus retains empty class I molecules in the ER (Jackson et al. 1994). It is only upon peptide loading that the fully assembled heavy chain/β_2m/peptide complex is released by calnexin for transportation to the cell surface.

Class II molecules also start their existence in the ER. The two chains, α and β, assemble and are bound by a chaperone-like molecule, the invariant chain [(Ii) (Cresswell 1994)]. This molecule has two functions; one is to direct the α,β-heterodimer to the class II loading compartment, which appears to be a specialized vesicle characterized by the presence of class II molecules. The second function of Ii is the prevention of premature peptide loading to class II molecules. The molecular interactions between Ii and the α,β-heterodimer preventing peptide binding are not completely sorted out; one possibility is an allosteric effect of Ii on the dimer such that the peptide binding groove is closed due to conformational change. The other possibility is that a particular stretch of the invariant chain binds into the groove and thereby competitively prevents the binding of peptides. This latter view is derived from the observation that Ii peptides, called CLIPs (class II-associated invariant chain peptides) are frequently found associated with immunoprecipitated class II molecules, and that CLIPs are especially abundant in cells with a defect in antigen processing. In any case, Ii is removed from the α,β-heterodimer in the class II loading compartment, or shortly before. The peptides loaded onto class II molecules can be derived not only from endocytosed protein but also from protein endogenous to the cells, especially membrane-bound proteins which have a chance to co-localize in the class II loading compartment. Finally, the peptide-loaded α,β-heterodimers are translocated to the cell surface.

The simplified view shown in Figure 1 suggests a strict separation of the processing pathways for class I and class II, respectively. There is strong evidence, however, for considerable cross-talk between the two pathways. Peptides derived from cytosolic proteins, for example, can be loaded onto class II molecules (Pinet et al. 1994). On the other hand, peptides derived from phagocytosed proteins can be loaded onto class I molecules, especially if the phagocytosed protein is aggregated (Pfeifer et al. 1993; Rock et al. 1993). Such side-lines of processing pathways deserve interest because they could be exploited for new strategies of immune intervention.

Methods of characterizing MHC/peptide interactions

The most seminal approach to gain information on the function of MHC molecules as peptide receptors is the X-ray analysis of MHC crystals (Stern and Wiley 1994). The two other principle methods are: 1) Biochemical isolation and study of naturally MHC-associated peptides, and 2) Binding studies with synthetic peptides. The latter two approaches are discussed below:

Analysis of natural MHC ligands

The diagram in Figure 2 gives an overview on the approaches used for isolation and analysis of MHC-associated peptides.

The major technical challenge is the small copy number of individual peptides. It is estimated that a cell presents well over 1000 different peptides on its 100000 or so copies of a given MHC allelic product. A few of these peptides are present in high copy number, that is, up to 10000 or more. By far the most ligands, however, occur in a much lower copy number, maybe even down to as low as one copy per cell.

The most sensitive means of detecting isolated peptides is the T-cell assay, which is able to detect peptides in the sub-picomolar range, at least as far as cytotoxic T cells are concerned (Rötzschke et al. 1990). Typically, a peptide-containing sample (e.g., a few µl of an HPLC fraction) is incubated in a total volume of 100 µl together with MHC-expressing, ^{51}Cr-labeled target cells. After some incubation time, e.g., 90 min, CTL are added, the supernatant is harvested 4 to 6 h later, and the relative radioactivity measured indicates the degree of target cell lysis. If the 100 µl volume used for target cell incubation has a concentration of 1 pM, the absolute amount of peptide is 100 attomol, a sensitivity not reached by any other method. The use of the CTL assay, of course, is limited to the detection of T-cell epitopes for which T cells are on hand: Viral antigens, minor H antigens, tumor-associated antigens, transfected model antigens, or antigens recognized by alloreactive T cells. On the other hand, peptide detection assays for class-II-restricted T cells appear to be less sensitive than for class I-restricted T cells.

The major shortcoming of the T-cell assay for peptide detection is that it does not give sequence information. However, the location of a T-cell epitope among HPLC-separated MHC ligands of an infected cell can allow identification of the peptide in combination with biochemical analysis such as Edman degradation or mass spectrometry. The first naturally processed viral T-cell epitopes indeed were identified by the combination of T-cell assay with mass spectrometry, comparison of the HPLC behavior of synthetic and natural peptides, or partially direct sequencing, using radiolabeled amino acids incorporated by virus-infected cells (Rötzschke et al. 1990; van Bleek and Nathenson 1990). A combination of these methods for identification of T-cell epitopes is only possible if the proteins of origin are known. Direct sequencing of HPLC fractions containing a T-cell epitope is rarely successful, namely, only in cases where the T-cell epitope happens to be a peptide highly abundant in that fraction. A marked improvement of sensitivity was brought about by an ingenious combination of HPLC, CTL assay, and mass spectrometry by Cox and co-workers (1994).

By far the most ligands known to date are not T-cell epitopes and these ligands were determined by direct sequencing, either by Edman degradation, or by mass spectrometry, or by a combination of the two methods. Detection limit of Edman degradation is about 1 pmol, that

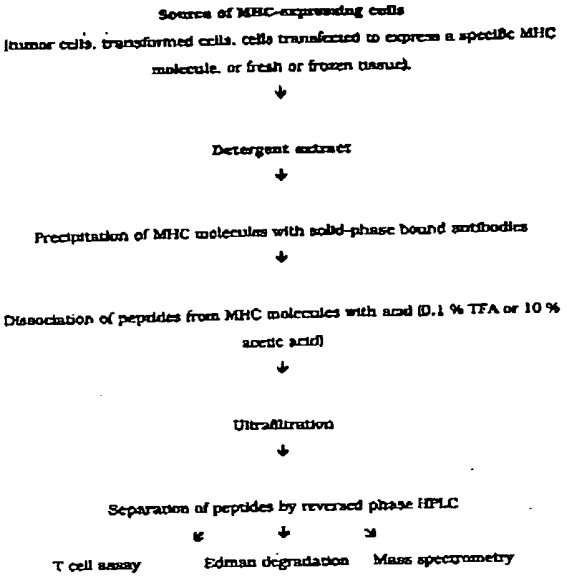

Fig. 2 Methods for analysis of MHC ligands

is, the equivalent of 6×10^9 cells for a peptide occurring in 100 copies per cell. Sequencing by tandem mass spectroscopy has been reported to be more sensitive – down to 30 fmol or less. It is, however, challenging to achieve this degree of sensitivity, so that, apart from the pioneering group of Hunt and co-workers (1992), not many other laboratories have come up with similar results.

A special application of Edman degradation is pool sequencing, that is, altogether-sequencing of the complex mixture of peptides eluted from a given MHC species (Falk et al. 1991b). Although disliked by purists, this approach allows one to determine the common characteristics of all peptides associated with a given MHC molecule, with relatively little effort. Pool sequencing of MHC class I ligands led to the discovery of the principle of allele-specific motifs, and allowed a large number of such motifs to be determined. The clear information that can be obtained from pool sequencing of class I ligands is made possible by their uniform length, e.g., 9 amino acids. But even for class II ligands, which can range in length from about 12 to 25 amino acids, pool sequencing is a valuable tool for gaining detailed information on motifs (Falk et al. 1994b).

It appears that the number of amino acids between the N-terminus of class II ligands and the first anchor varies by about three amino acids for the majority of ligands. For DR1, for example, the distance from the N-terminus to the first anchor of the majority of ligands is 5 ± 1 (Falk et al. 1994b). Thus, pool sequencing indicates a cluster at position 4, 5, and 6 for the anchor residues used, aromatic and aliphatic. Again for DR1, the next cluster stretches over

positions 7, 8, and 9, indicating the next anchor for aliphatic residues. The rough motif obtained by such interpretations – absolute position 5 set as relative position 1 to mark the first anchor – can then be complemented and worked out in depth by applying 1) alignment of natural ligands, 2) consideration of the pockets, as revealed recently by crystallography of a monopeptidic DR1 molecule (Stern et al. 1994), and 3) considerations of peptide binding assays. If all four sources of information are considered, a detailed picture of the degenerate (as compared with class I) peptide specificities of class II molecules can be obtained that should be useful for epitope predictions (Friede and coworkers, submitted).

2) Peptide binding assays

MHC/peptide binding assays have a history of leading to obsolete results. On the other hand, with our increasing knowledge of MHC structure and MHC/peptide interaction and specificity, it is possible to design straightforward peptide binding experiments to answer specific questions. A number of approaches can be used to measure peptide binding to MHC. The oldest method is as follows (Buus et al. 1987): MHC molecules are purified and incubated with radioactively labeled peptides. Then the mixture is subjected to a gel filtration column. MHC molecules with the radioactive peptide bound will elute in the exclusion volume, whereas free peptides will elute later. Thus, the amount of radioactivity in the exclusion volume is a measure for peptides bound to MHC. The binding behavior of other, unlabeled peptides can be tested via their capacity to inhibit binding of the radioactive peptide. A number of variations of this method have been used. For example, the radioactive label can be replaced by a fluorescent marker. Furthermore, MHC/peptide complexes can be separated from free peptides by gel electrophoresis, or upon binding of the MHC/peptide complex to solid phase with the help of antibodies. In the latter case, however, two different antibodies reactive with different sites of the MHC molecule are required, one for purification of the MHC molecule, the other for capturing the MHC/peptide complex from the reaction mixture.

Depending on the conditions, these kinds of peptide binding assays can be made very sensitive to detect even low-affinity peptide binding. This may result in problems of interpretations, since low-affinity binding might not be relevant for physiological MHC/peptide interactions.

A second type of binding assay depends on the stabilization of MHC class I molecules by bound peptides. Cells with a defect in antigen processing, for example, TAP-defective RMA-S cells, express only a low density of antibody-detectable MHC class I molecules on their surface, if cultured under normal conditions (37 °C). If such cells are incubated with peptides binding to the expressed class I molecules with high affinity, the latter are stabilized, and their surface density increases (Townsend et al. 1989). Since determination of class I surface density can be easily done by FACS analysis, this approach has been widely used. Since only few cell lines with transporter defects are known, the assay can only be used for MHC molecule expressed by such cells, e.g., H-2K^b or D^b for RMA-S cells. To study peptide binding for additional MHC-molecules the desired MHC molecule can be expressed in RMA-S or other TAP-defective cells upon gene transfection. The advantage of this MHC-stabilization assay is that it is rather insensitive and thus detects only peptides binding with high affinity that are likely to be physiologically relevant. Stabilization of MHC molecules by peptides can also be measured with purified MHC molecules.

For class II molecules, the binding of high-affinity peptides leads to a compact form of the MHC/peptide complex, as seen by SDS gel electrophoresis, whereas a peptide of lower affinity leads to a "floppy" form of class II molecules.

A very elegant approach for studying the peptide specificity of class II molecules has been developed by Hammer and co-workers (Sinigaglia and Hammer 1994). A peptide library is expressed by bacteriophages. From the peptide-expressing phages only those are selected which are able to bind to a given class II molecule. The peptide sequences expressed by the selected phages are then determined. With this approach, a peptide binding motif of HLA-DR1 has been established that is well reflected among the natural ligands, and can be well explained by the crystal structure of HLA-DR1.

MHC class I ligands and motifs

The main purposes for which this information will be useful are the prediction of T-cell epitopes within proteins of known sequences and the detailed analysis of peptide/MHC interaction. For epitope prediction it is important not to consider just the basic motif of a given MHC molecule, since the non-anchor positions of peptides could also contribute considerably to the interaction with MHC. This is evident from the preferences seen for certain residues at non-anchor-positions in pool sequencing data, from the interaction of such residues with MHC sites as seen in crystals (Madden et al. 1993; Zhang et al. 1992; Fremont et al. 1992), and from detailed binding studies showing that certain residues at a given peptide position can be detrimental for binding (Ruppert et al. 1993; Kast et al. 1994; Parker et al. 1994).

The basic approach to search a protein sequence for an epitope fitting to a given class I molecule is as follows. First, the sequence is screened for stretches fitting to the basic anchor motif (2 anchors in most cases), whereby allowance should be made for some variation in peptide length as well as in anchor occupancy. If a motif, for example, calls for 9mers with I or L at the end, 10mers with a fitting C-terminus should be considered as well, and other aliphatic residues at the C-terminus, like Val or Met, should also be considered. In this way, a list of candidates will be obtained. These are now inspected for having as many non-anchor residues as possible in common with

ligands already known, or with the residues listed among the "preferred residues" or "others" on top of each motif Table. If possible, a binding assay can be performed at this stage to exclude weak binders which occur frequently among peptides conforming to a basic motif. If a detailed study on peptide binding requirements is available, the candidates can also be screened for non-anchor residues detrimental or optimal for binding (Ruppert et al. 1993; Kast et al. 1994; Romero et al. 1991; Ebert et al. 1993). One should keep in mind, however, that pure peptide binding motifs can be misleading in the search for natural ligands, since other constraints, such as enzyme specificity during antigen processing and specificity of transporters or chaperones, are likely to contribute to ligand identity in addition to the MHC binding specificity.

A careful consideration of the pocket structure of the MHC molecule of interest can also be useful for epitope prediction (Falk and Rötzschke 1993). For the P1 residue, for example, preferences can be explained by the residues contributing to the P1 contact site (Falk et al. 1995a, c). Since the MHC residues contributing to the different contact sites can differ among MHC molecules, such considerations should be held with caution, however (Guo et al. 1993). Computer modeling of the MHC molecule in question can be of help here.

The use of allele-specific peptide motifs is limited to a certain extent by exceptional ligands not fitting to a motif, e.g., Frumento and co-workers (1993) and Mandelboim and co-workers (1994). Such ligands will be missed by epitope predictions based on allele-specific motifs. It is not clear as yet how frequently this happens. In most cases, natural ligands will fit to the motifs, whereby substitutions of anchor residues with residues of similar chemistry (e.g., one aliphatic residue for another) and length variations are not infrequent and should be considered. A special case is the mouse H-2M3 molecule. A complete motif is not known, except that this molecule is specialized to present N-formylated peptides of bacterial or mitochondrial origin (Fischer-Lindahl 1991; Shawar et al. 1991).

MHC class II ligands and motifs

The long-awaited X-ray analysis of class II molecules has given us invaluable insight into peptide/class II interactions (Brown et al. 1993; Stern et al. 1994). Especially the detailed information on the 5 DR1-pockets accommodating anchoring side chains of one particular ligand, influenza haemagglutinin 306-318, provided a structural basis for the previously worked out peptide ligand motif of DR1 molecules (Rötzschke and Falk 1994; Sinigaglia and Hammer 1994). Moreover, pocket spacing and structure, as found for this one particular DR1/peptide complex, can be used to deduce the putative interaction for other DR1-peptide complexes and even for some other class II molecules. We found it particularly useful to evaluate pool sequencing data under the aspect of the expected pocket structure (Friede and co-workers, submitted; Schild and co-workers, submitted). Combined with the alignment of individual class II ligands, this approach is a powerful tool to determine allele-specific class II peptide motifs, as we have exercised recently for several closely related DR4 subtypes (Friede and co-workers, submitted).

The general picture for allele-specific class II motifs emerging is as follows. A stretch of nine amino acids, on average starting at absolute positions 3 to 5 of natural ligands, is determined by the respective allele-specific motif, corresponding to the peptide portion embedded in the MHC groove. The first position of this nonamer stretch, P1, represents a hydrophobic anchor for all class II ligand motifs known so far. Anchoring of the hydrophobic P1 side chain in the respective class II pocket appears to be particularly intensive, as impressively illustrated by the deep pocket seen in the monopeptidic DR1 crystal. The importance of P1 side chains is also indicated by the striking influence of P1 on peptide binding, and by the significant clustering of hydrophobic residues at cycles 3 to 5 of self-peptide pools. In addition to P1, several other anchors follow up to P9. For DR1, these are at P4, P6, P7, and P9, as indicated by structural data, whereby the specificity of P7 is somewhat degenerate and escapes detection in binding assays or natural ligand analysis. For several other class II molecules, the same anchor spacing – P1, P4, P6, P7, P9 – is compatible with ligand motif data. DR2, DR3, and DR4 motifs as well as H-2E motifs fall into this category. Other molecules, like DR5, DPw4, and DQ7 appear to have slightly different anchor spacing, e.g., the second anchor at P3, or an anchor at P5. Allele-specific differences can occur at each of the anchor positions, although differences of P1 specificity in HLA-DR molecules are limited by the β86Gly/Val polymorphism. More pronounced allele-specific differences are found for P4, P6, and P9, respectively. Charge differences are particularly evident: P4 of DR17, for example, requires Asp, whereas P4 of DR4Dw10 does not tolerate Asp or Glu but prefers basic or hydrophobic residues. P9, on the other hand, prefers hydrophobic residues for DR1 but negative charges for DR4Dw15 and positive charges for H-2E^k. Interestingly, charge differences in polymorphic stretches of class II molecules (probably reflecting counter charges for charged anchors) have been found to be associated with autoimmune diseases (Gregersen et al. 1987; Khalil et al. 1990; Todd et al. 1987).

Epitope prediction of class II ligands within a protein is not as easy as with class I, because the anchors, or interaction sites, are more degenerate in their specificity. The first step should be to pick out the most allele-specific anchor beyond P1, for example, P4 of DR17, P6 of DR1, or P9 of H-2E^k or DR4Dw15. The selection of nonamer sequences fitting to P1 and the other anchor of the respective motif is then further examined for adherence to the additional anchors. The resulting collection of nonamer stretches might then be inspected for adherence to the putative processing motif XPXX in the flanking regions (Rötzschke and Falk 1994). A quantitative ranking of the contribution of each amino acid residue at almost every position has been determined in an elegant approach by

Hammer and co-workers (1994) for DR4, which led to highly accurate predictions of good DR4 binders.

Technical notes

We have tried to put together all the motifs and natural ligands we were aware of. Due to the flood of data emerging in the past years, however, we anticipate that some information has been overlooked. We apologize in advance to those authors whose work was inadvertently not adequately covered.

In case of those class II ligands occurring as nested sets, we included only one or a few members of the set in many cases.

An X in peptide sequences stands for an undetermined amino acid. However, if the peptide sequence has been determined by mass spectometry, as is the case for the peptides reported by Hunt and co-workers (1992a, b), X stands for either Leu or Ile (which have the same mass). Lowercase letters in peptide sequences indicate residue determination of lower confidence.

As far as T-cell epitopes are concerned, only those have been selected which are likely to be naturally processed; criteria for judgement are to be found in Stevanović and Rammensee (1995). From the numerous class II motifs that have been published, we selected the more convincing ones, that is, those compatible with the class II structure. Due to the variable number of amino acids between the N-terminus and the first anchor of peptides, alignment of ligands or T-cell epitopes to class II motifs is always arbitrary, unless a structural analysis has been performed. For the class II molecules without reasonable motifs, a list of the published ligands is provided, without any attempt at alignment.

If you wish to have your motifs or ligands included in forthcoming listings, please send us reprints (no preprints) of the work describing them. We would also appreciate any comments on errors and omissions, as well as suggestions for improvements.

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Table 1 Mouse class I motifs
A H-2K^d

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues		Y F							I L V		a
Preferred residues			N I L	P	M	K F	T N				
Others	K A R S V T		A H V R S F E Q K M T	A E S D H N	V N D I L S T G	H I M Y V R L	P H D E Q S	H E K V F R			
Examples for ligands	T	Y	Q	R	T	R	A	L	V*	Influenza A NP 147–154	b, c
	S	Y	F	P	E	I	T	H	I	Tyrosine kinase JAK1 355–363	a, d
	K	Y	Q	A	V	T	T	T	L*	Tum-P198 14–22	a, e, f
	G	Y	K	D	G	N	E	Y	I*	Lysteriolysin O 91–99	g
	K	Y	G	V	S	V	Q	D	I*	L. monocytogenes p60 217–225	h
	G	Y	L	G	Q	V	T	X	I	Unknown	u
	S	F	V	D	T	R	T	L	L	Collagen 1 α2 4–12	u
T-cell epitopes	L	Y	Q	N	V	G	T	Y	V	Influenza JAP HA 204–212	i
	T	Y	V	S	V	G	T	S	T L	Influenza JAP HA 210–219	i
	V	Y	Q	I	L	A	I	Y	A	Influenza JAP HA 523–531	i
	I	Y	A	T	V	A	G	S	L	Influenza JAP HA 529–537	i
	T	Y	V	S	V	S	T	S	T L	Influenza A HA 210–219	k, l
	I	Y	S	T	V	A	S	S	L	Influenza A HA 518–526	k
	R	Y	L	E	N	G	K	E	T L	HLA-A24 170–179	a, m
	R	Y	L	K	N	G	K	E	T L	HLA-Cw3 170–179	a, m
	S	Y	I	P	S	A	E	K	I	P. berghei CSP 252–260	u
	S	Y	V	P	S	A	E	Q	I	P. yoelii CSP 281–289	o
	S	Y	I	G	S	I	N	N	I	RSV M2 82–90	p
	D	Y	A	T	L	G	V	G	V	HSV-1 ICP27 448–456	q
	L	Y	R	T	F	A	G	N	P R A	HSV-1 ICP27 322–332	q
	T	Y	K	D	T	V	Q	L		Polio VP1 111–118	r
	F	Y	D	G	F	S	K	V	P L	Polio VP1 208–217	r
	A	Y	I	S	S	G	S	S	T L	Human Ig VH 49–58	l
	N	Y	D	N	A	G	T	N	L	P. falciparum CSP 39–47	s
	K	Y	L	K	K	I	K	N	S L	P. falciparum CSP 333–342	s
	K	Y	L	K	I	K	H	L	L	APC frameshift	t

* Also a T-cell epitope

References:
a: Falk et al. 1991 b; b: Rötzschke et al. 1990; c: Falk et al. 1991 a; d: Harpur et al. 1993; e: Sibille et al. 1990; f: Wallny et al. 1992; g: Pamer et al. 1991; h: Pamer 1994; i: Braciale et al. 1987; k: Kurwano et al. 1988; l: Cao et al. 1994; m: Maryanski et al. 1986; n: Romero et al. 1989; o: Weiss et al. 1990; p: Kulkarni et al. 1993; q: Banks et al. 1993; r: Kumbuddin et al. 1992; s: Blum-Tirouvanziam et al. 1994; t: Townsend et al. 1994; u: Reich et al. 1994

Table 1 (Continued)
B H-2D^d

	Position									Source	R
	1	2	3	4	5	6	7	8	9		a
Anchor or auxiliary anchor residues		G	P		R K				I L F		
Other preferred residues				D E Q		N I L	D E				
Examples for ligands	K	G	P	I	T	V	Q	I		Unknown	b
	V	G	P	Q	K	N	E	N	L	Unknown	b
	S	G	P	R	R	X	I	X	L	Homol. mRNA CD40 mouse	b
	A	G	P	D	K	T	E	K	X	Unknown	b
	K	G	P	D	R	G	N	E	F	Homol. metalloproteinase 2 inhibitor	b
	I	G	P	E	K	G	H	N	L	Homol. hypoxanthine phosphoribosyl-transferase	b
	D	G	P	V	R	E	H	N	L	Homol. urease canavalia ensiformis	b
	K	G	P	E	R	X	N	G	L	Unknown	b
	S	G	P	E	R	G	E	K	L	Homol. proliferating cell nucleolar antigen P40	b
	D	G	P	V	R	G	I	S	I	Homol. ribosomal protein S17 rat	b
	N	G	P	Q	R	I	Y	N	L	Unknown	b
	S	G	P	V	A	L	V	N	F	Unknown	b
	I	G	P	N	R	A	F	N	F	I	Unknown	b
	S	G	P	E	R	L	L	S	X	Y	Homol. heterog. nucl$_1$RNP complex K	b
	V	G	P	S	G	K	Y	F	I	L	Unknown	b
	F	G	P	Y	K	L	N	R	L		Homol. feline leukemia virus envelope polyprotein	b
	F	G	P	L	K	F	N	V	L	T	Unknown	b
	A	G	P	D	R	F	I	X	X	M	Unknown	b
	F	G	P	Y	R	F	Y	V	L	T	Unknown	b
	S	E	Q	D	L	N	F				Unknown	b
	S	X	H	K	E	Q	P	A	T		Homol. transforming protein spi-1 human	b
	S	X	P	K	T	D	X	Q	T	L	Homol. insulin receptor precursor	b
T-cell epitopes		G	P	P	H	S	N	N	F	G Y	Tum-P35B 4–13	a
	R	G	P	G	R	A	F	V	T	I	HIV gp160 318–327	f
	L	M	G	Y	I	P	L	V	G	A	HCV core 133–142	c

References:
a: Falk and co-workers, unpublished; b: Corr et al. 1993; c: Szikora et al. 1993; d: Takahashi et al. 1988; e: Shirai et al. 1994; f: Bergmann et al. 1993b

Table 1 (Continued)
C H-2L^d

	Position 1	2	3	4	5	6	7	8	9		Source	Ref.
												a, b, c
Anchor residues		P S							F L M			
Other preferred residues			G Q M L	T	T	I K F	F	Q N				
Examples for ligands	Y	P	H	F	M	P	T	N	L*		MCMV pp 89 168–176	d
	L	S	P	F	P	F	D	L*			OGDH 105–112	e
V A I T R I E Q	L	S	P	F	P	F	D	L*			OGDH 97–112	e
	X	P	L	E	A	N	Y	Q	X	F	Unknown	c
	A	P	Q	P	G	M	E	N	F		Unknown	c
	Q	P	Q	R	G	R	E	N	F		Unknown	c
	X	P	Q	P	G	R	E	Q			Unknown	c
	X	P	Q	P	N	L	Y	Q	L		Unknown	c
	X	P	A	X	A	Y	P	Y			Unknown	c
	Y	P	N	V	N	I	H	N	F		Unknown	c
	X	P	Q	K	A	G	G	F	L	M	Phosphoglycerate kinase 180–189	c
T-cell epitopes	R	P	Q	A	S	G	V	Y	M		LCMV NP 118–126	f, g
	I	S	T	Q	N	H	R	A	L		Tumor antigen P91A 12–20	h
	L	P	Y	L	G	W	L	V	F		Tumor antigen P815 35–43	i
	A	P	T	A	G	A	F	F	F		JHMV Nucleocapsid 318–326	k
	Y	P	A	L	G	L	H	E	F		Measles NP 281–289	l
	T	P	H	P	A	R	I	G	L		E. coli β-gal. 876–884	m
	D	P	V	I	D	R	L	Y	L		Measles HA 343–351	n
	S	P	G	R	S	F	S	Y	F		Measles HA 544–552	n

* Also a T-cell epitope

References:
a: Falk et al. 1991b; b: Falk and co-workers, unpublished; c: Corr et al. 1992; d: Reddehase et al. 1989; e: Udaka et al. 1992; Udaka et al. 1993; f: Whitton et al. 1989; g: Schulz et al. 1991; h: Lurquin et al. 1989; i: Lethé et al. 1992; k: Bergmann et al. 1993a; l: Beauverger et al. 1993; m: Gavin et al. 1994; n: Beauverger et al. 1994

Table 1 (Continued)
D H-2K^b

	Position									Source	Ref.
	1	2	3	4	5	6	7	8			
Anchor or auxiliary anchor residues			Y		F Y			L M I V			a
Other preferred residues	R I L S A	N	P	R D E K T		T I E S	N Q K				
Examples for ligands	R	G	Y	V	Y	Q	G	L*		VSV NP 52–59	b
	S	I	I	N	F	E	K	L*		Ovalbumin 258–276	a, c, d
	H	I	Y	E	F	P	Q	L		Unknown	n
T-cell epitopes	I	I	Y	R	F	L	L	I		Rotavirus VP7 33–40	e
	S	S	I	E	F	A	R	L		HSV glycoprotein B 498–505	f
F	A	P	G	N	Y	P	A	L		Sendai virus NP 324–332	g, h
	K	S	P	W	F	T	T	L		MuLV p15E 574–581	i, k
	V	G	P	V	F	P	P	G	M	Rotavirus VP6 376–384	l
Y	S	G	Y	I	F	R	D	L		Rotavirus VP3 585–593	l
	F	E	Q	N	T	A	Q	A+		MUT 2 tumor antigen	m
	F	E	Q	N	T	A	Q	P+		MUT 1 tumor antigen	m

* Also a T-cell epitope
+ One of these peptides was found in a total cell extract of K^b-expressing tumor cells

References:
a: Falk et al. 1991b; b: van Bleek and Nathenson 1990; c: Rötzschke et al. 1991; d: Carbone et al. 1988; e: Franco et al. 1993; f: Bonneau et al. 1993; g: Kast et al. 1991; h: Schumacher et al. 1991; i: Sijts et al. 1994; k: White et al. 1994; l: Franco et al. 1994; m: Mandelboim et al. 1994; n: Wallny 1992

Table 1 (Continued)

E H-2D[d]

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues					N				M I		a
Preferred residues		M	I L P V	K E Q V		L F					
Others	A N I F P S T V	A Q D	G	D T		A Y T V M E Q H I K P S	D E Q V T Y	F H K S Y			
Examples for ligands	A	S	N	E	N	M	E	T	M*	Influenza A34 NP 366–374	a, b, c
	I	Q	V	G	N	T	R	T	I*	Yersinia YOP 51 249–257	n
T-cell epitopes	A	S	N	E	N	M	D	A	M	Influenza A68 NP 366–374	d
	S	A	I	N	N	Y	A	Q	K L	SV 40 T 206–215	e, o
	C	K	G	V	N	K	E	Y	L	SV 40 T 223–231	e, o
	Q	G	I	N	N	L	D	N	L	SV 40 T 489–497	e, o
	S	G	P	S	N	T	P	P	E I	Adenovirus 5 E1A 234–243	f
	F	Q	P	Q	N	G	Q	F	I	LCMV NP 396–404	g
	S	G	V	E	N	P	G	G	Y C L	LCMV GP 276–286	h
	K	A	V	Y	N	F	A	T	C G	LCMV GP 33–42	i, k
	R	A	H	Y	N	I	V	T	F	HPV16 E7 49–57	l
	N	N	L	D	N	L	R	D	Y (L)	SV 40 T 492–500 :501)	m

* Also a T-cell epitope

References:
a: Falk et al. 1991b; b: Rötzschke et al. 1990; c: Townsend et al. 1986; d: Cerundolo et al. 1991; e: Deckhut et al. 1992; f: Kast et al. 1989; g: Yanagi et al. 1992; h: Oldstone et al. 1988; i: Oldstone et al. 1993; k: Klavinskis et al. 1990; l: Feltkamp et al. 1993; m: Alsheikly 1994; n: Sturnbach and Bevan 1994; o: Tevethia et al. 1990

Table 1 (Continued)
F H-2K^k

	Position									Comments	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues		E						I	I	C-terminus at P8 or P9	a, b.
Preferred residues	V F	D	K N Y M Q I L F P H T	L	A G P T V F S	N K H	T				
										Source	
Examples for natural ligands	H	E	T	T	F	N	S	I		β Actin 275–282	k
	D	D	H	R	A	G	K	I		S24 ribosomal protein 53–60	k
	Y	E	D	T	G	K	T	I		Unknown	k
	K	E	M	K	A	K	V	I		Homol. T cell transcript. factor 1	k
	E	E	E	P	V	K	K	I		Hn RNP C protein 84–91	k
	S	E	I	V	G	K	R	I		S7/S8 ribos. protein 137–144	k
	S	E	G	G	S	H	T	I		H-2D^k 112–119	k
	D	E	R	T	V	R	K	I		Unknown	k
	E	E	D	P	V	K	K	V		CArG bind. factor A 209–216	k
	E	A	Y	L	G	K	K	V		BiP 158–165	k
T-cell epitopes	F	E	A	N	G	N	L	I		Influenza A HA 259–266	c, i
	I	E	G	G	W	T	G	M	I	Influenza A HA 10–18	c, i
	S	D	Y	E	G	R	L	I		Influenza A NP 50–57	d, l
	F	E	S	T	G	N	L	I		Influenza JAP HA 255–262	e
	S	E	F	L	L	E	K	R	I	SV 40 T 560–568	f
	Y	E	N	D	I	E	K	K	I	P. falciparum CSP 375–383	g
	D	E	L	D	Y	E	N	D	I	P. falciparum CSP 371–379	g
	T	E	M	E	K	E	G	K	I	HIV-1 RT 206–214	h
	V	E	A	E	I	A	H	Q	I	Rabies NS 197–205	i
	E	E	G	A	I	V	G	E	I	Influenza A NSI 152–160	a

References:
a: Cossins et al. 1993; b: Norda et al. 1993; c: Gould et al. 1991; d: Bastin et al. 1987; e: Sweetser et al. 1989; f: Rawle et al. 1988; g: Kumar et al. 1988; h: Hosmalin et al. 1990; i: Larson et al. 1991; Gould et al. 1987; k: Brown et al. 1994; l: Gould et al. 1989

G H-2K^{bm1}

	Position								Source	Ref.
	1	2	3	4	5	6	7	8		
Anchor or auxiliary anchor residues		E						I		a
Other preferred residues		Q G P	K N Q G M P Y	P	A R K		R Y			

References:
a: Norda et al. 1993

Table 1 (Continued)

H Qa-2	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		a, b
Anchor or auxiliary anchor residues		M L Q	N I L		V I	K M I	H		L I F		
Other preferred residues	K A E Q		T	P E A G K S D	L T E H M F Y	L F N Y	R	E Q N D K S T R			
Examples for ligands	K A K A K V R K A G I A S K K S Y D	Q L Q G L Q S L M Q L Q K M Q V E L	N A N L I N N T L L M N I L L L S L	P E P L K X G G A X E P K P I D P G	I L T G V T Q I T V H V T V D L T	A P V M Y M V K Y X I L V E T L M L	H H H R H H H H H H H Y R H Y A X H	Q E H S P M E K K K Q E N H L F N	L L L G L I L L L L L L I M L L L L	L	Unknown Unknown Unknown Unknown Unknown Unknown Unknown Cofilin 127–135 Unknown Unknown Ribosomal protein L19 137–145 Unknown Unknown Unknown Unknown Unknown Unknown Unknown	b b b b b b b b b b b b b b b b b b b

References:
a: Rötzschke et al. 1993; b: Joyce et al. 1994

I Selected other T-cell epitopes													Comments	Ref.
MHC	Sequence													
H-2D^k		R	R	K	G	K	Y	T	G	L			T cell epitope of LEC-A	a
H-2M3	fM		F	F	I	N	I	L	T	L	L	V	ND1α 1–17	b
	fM		F	F	I	N	A	L	T	L	L	V	ND1β 1–17	b

References:
a: de Bergeyck et al. 1994; b: Fischer Lindahl 1991

Table 2 HLA-A motifs
A HLA-A1

	Position													Source	Ref
	1	2	3	4	5	6	7	8	9						
Anchor or auxiliary anchor residues		T S	D E	P			L		Y						a, b, f, i
Other preferred residues		L		G I	G N Y	G V I									
Examples for ligands	A	T	D	F	K	F	A	M	Y					Cyclin-like protein 135–143	a, i
	I	A	D	M	G	H	L	K	Y					Proliferation cell nuclear antigen 241–249	a, b
	M	I	E	P	R	T	L	Q	Y					Ribosomal protein S16 40–48	a, b
	Y	T	S	D	Y	F	I	S	Y					Ets-1 154–162	a, i
	L	T	D	P	G	V	L	D	Y					Unknown	a
	V	S	D	I	V	G	P	D	G	L	V	Y		Fibrillarin 177–188	a, b
	Y	T	D	Y	G	G	L	I	F	N	S	Y		Cytochrome C oxidase II	a, i
	Q	S	E	D	G	S	H	T	I	Q	I	M	Y	HLA class I α chain 111–123	a
	Y	L	D	D	P	D	L	K	Y					Cytosine methyl transferase 238–246	i
	S	T	D	H	I	P	I	L	Y					Fructose-6-amino transferase 217–225	i
	D	S	D	G	S	F	F	L	Y					IgG4 279–287	i
	G	T	D	E	X	R	N	X	Y					Unknown	i
	V	S	D	P	Y	N	X	K	Y					Unknown	d, i
	V	A	D	K	V	H	X	M	Y					Unknown	i
	Y	T	A	V	V	P	L	V	Y					J-chain 102–110	i
	Y	T	N	P	Q	F	N	V	Y					Unknown	i
	E	T	X	X	P	D	W	S	Y					Unknown	i
	F	T	D	V	N	S	X	X	R	Y				Unknown	i
	S	S	E	Q	T	F	M	Y	Y					Ornithine decarboxylase 309–317	b
	S	T	E	P	V	N	I	L	Y					Unknown	b
	G	T	D	P	G	V	L	I	Y					Unknown	b
	S	T	E	P	P	M	L	N	Y					Unknown	b
	S	L	E	P	Q	R	T	Q	Y					Unknown	b
	F	T	E	V	S	I	R	K	Y					Unknown	b
	K	F	D	P	V	N	L	V	Y					Unknown	b
	A	V	D	P	G	G	M	Y	S					Unknown	b
	F	G	S	G	A	R	D	X	Y					Unknown	b
	Y	X	E	P	Q	F	L	T	Y					Unknown	b
	A	X	I	P	A	F	I	N	Y					Unknown	b
	I	T	E	D	M	G	H	L	K	Y				Unknown	f
	E	T	D	X	X	X	D	R	S	E	Y			Unknown	i
T-cell epitopes	E	A	D	P	T	G	H	S	Y					MAGE-1 161–169	c, k
	V	S	D	G	G	P	N	L	Y					Influenza A PB1 591–599	b, f
	C	T	E	L	K	L	S	D	Y					Influenza A NP 44–52	f
	E	V	D	P	I	G	H	L	Y					MAGE-3	g, h

References:
a: Falk et al. 1994c; b: Di Brino et al. 1993b; c: Sette et al. 1994; d: Engelhard 1994; e: Traversari et al. 1992; f: DiBrino et al. 1994; g: Gaugler et al. 1994; h: Celis et al. 1994; i: Kubo et al. 1994; k: Van der Bruggen et al. 1991

Table 2 (Continued)
B HLA-A*0201

	Position														Source	Ref.
	1	2	3	4	5	6	7	8	9							
Anchor or auxiliary anchor residues		L M				V			V L							a
Preferred residues				E K				K								
Other residues	I L F K M Y V		A Y F P M S R	G P D T	I K Y N G F V H	I L T	A Y H	E S								
Examples for ligands	S	L	L	P	A	I	V	E	L						Protein phosphatase 2A 389–397	b
	Y	L	L	P	A	I	V	H	I						ATP-dependent RNA Helicase 148–156	b
	T	L	W	V	D	P	Y	E	V						B-cell translocation gene 1 protein 103–111	b
	S	X	P	S	G	G	X	G	V						Unknown	b
	G	X	V	P	F	X	V	S	V						Unknown	b
	S	X	X	V	R	A	X	E	V						Unknown -	b
	K	X	N	E	P	V	X	X	X						Unknown	b
	A	L	W	G	F	F	P	V	X*						Unknown mouse protein	b, e
	L	L	D	V	P	T	A	A	V						IP-30 signal sequence 27–35	b
L	L	L	D	V	P	T	A	A	V						IP-30 signal sequence 26–35	b, c
L	L	L	D	V	P	T	A	A	V	Q	A				IP-30 signal sequence 26–37	b, c
L	L	L	D	V	P	T	A	A	V	Q					IP-30 signal sequence 26–36	c
	V	L	F	R	G	G	P	R	G	L	L	A	V	A	SSR α signal sequence 12–25	d, e
	M	V	D	G	T	L	L	L	L						HLA-E signal sequence 1–9	b
	Y	M	N	G	T	M	S	Q	V*						Tyrosinase 369–377	f, g, h, i
	M	L	L	S	V	P	L	L	L	G					Calreticulin signal sequence 1–10	c
	S	L	L	G	L	L	V	E	V*						Unknown	l
	A	L	L	P	P	I	N	I	L*						Unknown	l
	T	L	I	K	I	Q	H	T	L*						Unknown	l
	A	L	I	V	G	X	N	D	D*						Unknown	l
	H	L	I	D	Y	L	V	T	S*						Carboxypeptidase M 91–99	l
	I	L	A	P	P	V	V	K	L	L	V*				Unknown	l
	A	L	F	P	Q	L	V	I	L*						Unknown	l
	G	I	L	G	F	V	F	T	L-						Influenza matrix protein 58–66	a, k, o, y, z
T-cell epitopes	I	L	K	E	P	V	H	G	V						HIV-1 RT 476–484	a, c, j
	I	L	G	F	V	F	T	L	T	V					Influenza matrix protein 59–68	a, k
	L	L	F	G	Y	P	V	Y	V						HTLV-1 tax 11–19	o
	G	L	S	P	T	V	W	L	S	V					Hepatitis B sAg 348–357	m
	W	L	S	L	L	V	P	F	V						Hepatitis B sAg 335–343	m
	F	L	P	S	D	F	F	P	S	V					Hepatitis B Nucleocapsid 18–27	n
	C	L	G	G	L	L	T	M	V						EBV LMP2 426–434	p
	F	I	A	G	N	S	A	Y	E	Y	V				HCMV glycoprotein B 618–628	m
	K	L	G	E	F	Y	N	Q	M	M					Influenza B NP 85–94	q
	K	L	V	A	L	G	I	N	A	V					HCV-1 1406–1415	r
	D	L	M	G	Y	I	P	L	V						HCV core 132–140	s
	R	L	V	T	L	K	D	I	V						HPV 11 E7 4–12	t
	M	L	L	A	L	L	Y	C	L						Tyrosinase 1–9	f, g
	A	A	G	I	G	I	L	T	V						Melan A/Mart 1	w, x
	Y	L	E	P	G	P	V	T	A						pmel 17/gp100	u
	I	L	D	G	T	A	T	L	R	L					pmel 17/gp100	v

* Class I ligands allocated to A2 by motif. + Also a T-cell epitope

References:
a: Falk et al. 1991b; b: Hunt et al. 1992; c: Henderson et al. 1992; d: Wei and Cresswell 1992; e: Henderson et al. 1993; f: Wölfel et al. 1994; g: Robbins et al. 1994; h: Brichard et al. 1993; i: Engelhard et al. 1993; j: Walker et al. 1989; k: Gotch et al. 1988; l: Harris et al. 1993; m: Nayersina et al. 1993; n: Bertoletti et al. 1993, 1994; o: Utz et al. 1992; p: Lee et al. 1993; q: Robbins et al. 1989; r: Chisari and co-workers, personal comm.; s: Shirai et al. 1994; t: Tarpey et al. 1994; u: Cox et al. 1994; v: Kawakami et al. 1994b; w: Coulie et al. 1994; x: Kawakami et al. 1994a, c; y: Falk et al. 1994a; z: Bednarek et al. 1991

Table 2 (Continued)

C HLA-A*0205

	Position									Source	Re
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		V L I M				I V L A			L		a
Other preferred residues		Q	Y P F I	G E D K N	Y V L I	T	Q	K			

References:
a: Rötzschke et al. 1992

D HLA-A3

	Position										Source	Ref.
	1	2	3	4	5	6	7	8	9	10		
Anchor or auxiliary anchor residues		L V M	F Y			I M F V L	I L M F		K Y F	K		a, b.
Other preferred residues	I				I P V K	T		Q S T K				
Examples for ligands	K	X	F	K	M	I	L	R	K		Unknown	a
	K	L	F	K	N	I	L	Y	K		Unknown	a
	Y	L	X	V	R	X	A	X	i	V	Unknown	a
	K	L	H	K	Q	R	A	K	S		Unknown	a
	S	L	F	K	Q	V	V	T	K		Unknown	a
	K	X	F	V	K	X	L	X	Y		Unknown	a
	S	L	F	N	T	H	L	X	K		Unknown	a
	T	L	A	N	D	X	V	V	P		Unknown	a
	G	I	F	A	X	X	X	V	K	A	Unknown	a
	T	X	F	V	K	X	L	X	Y		Unknown	a
	S	L	F	D	H	I	L	X	K	H	Unknown	a
	K	L	F	K	V	V	X	N	Y		Unknown	a
	K	L	Y	E	K	V	Y	T	Y	K	Unknown	a
	K	L	F	N	I	M	V	T	Y		Unknown	a
	K	L	F	E	K	V	F	S	Y		Unknown	a
	K	L	F	K	V	T	F	A	Y		Unknown	a
	G	L	F	P	X	Q	F	X	Y		Unknown	a
	S	L	F	E	L	V	F	D	Y		Unknown	a
	S	L	X	E	K	T	F	e	Y		Unknown	a
	S	L	H	K	Y	X	f	T	Y		Unknown	a
	K	M	F	N	I	T	v	N	Y		Unknown	a
	K	L	F	V	K	V	y	M	Y		Unknown	a
	K	I	V	R	K	P	G	A			Unknown	a
T-cell epitopes	R	L	R	D	L	L	L	I	V	T	R	HIV-1 env gp41 768–778	c
	Q	V	P	L	R	P	M	T	Y	K		HIV-1 nef 73–82	d
	T	V	Y	Y	G	V	P	V	W	K		HIV-1 env gp120 36–45	e
	R	L	R	P	G	G	K	K	K			HIV-1 gag p17 20–29	c
	I	L	R	G	S	V	A	H	K			Influenza NP 265–273	f

References:
a: DiBrino et al. 1993a; b: Maier et al. 1994; c: Takahashi et al. 1991; d: Koenig et al. 1990; e: Venet and Walker 1993; f: DiBrino et al. 1993b; g: Kubo et al. 1994

Table 2 (Continued)

E HLA-A*1101

	Position											Source	Ref.
	1	2	3	4	5	6	7	8	9	10	11		
Anchor or auxiliary anchor residues		V I F Y	M L F Y I A				L I Y V F		K	K	K		a, b, c
Other preferred residues	A	T	N D E Q	P G D E K	P I F V M	I V M		R K N E Q	R D	R	R		
Examples for ligands	A	V	M	R	P	E	A	E	K	R	K	Unknown	b
	A	V	I	L	P	P	L	S	P	Y	F K	HSB 66 EST 18–29	b
	A	S	F	D	K	A	K	L	K	K		Thymosin β-10 11–20	b
	G	Q	Y	G	N	P	L	N	K			Cattle metalloproteinase 19–27	b
	G	V	M	P	S	H	F	S	R			Ribosomal protein S19 93–101	b
	Y	F	D	P	A	N	G	K	F	S	K	Elongation factor 2 265–275	b
	A	T	A	G	D	G	L	I	E	L	R K	Prohibitin (rat) 229–240	b, c
	S	T	Y	Y	G	S	F	V	T	R		Unknown (also presented by A33)	a, b
	S	V	L	N	L	V	I	V	K			Ribosomal protein S6 107–115	c
	K	V	V	N	P	L	F	E	K			Ribosomal protein L7A 25–33	c
	R	T	Q	N	V	L	G	E	K			Ribosomal protein S3 54–62	c
	G	T	M	T	T	S	X	Y	K			Unknown	c
	A	S	F	D	K	A	K	L	K			Thymosin β-10 11–19	c
	A	A	M	X	D	T	V	V	F	K		Unknown	c
	R	V	E	Q	A	V	E	S	M	V	K	Unknown	c
T-cell epitope	I	V	T	D	F	S	V	I	K			EBNA 4 416–424	a, d

References:
a: Zhang et al. 1993; b: Falk et al. 1994c; c: Kubo et al. 1994; d: Gavioli et al. 1993

F HLA-A24

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		Y			I V	F			I L F		a
Other preferred residues			N E L M P G	D P			Q N	E K			
Examples for ligands	K	Y	P	E	N	E	F	L	L	Protein phosphatase 1 113–121	b
	Y	Y	E	E	Q	H	P	E	L	NK/T-cell activation protein 107–115	b
	A	Y	V	H	M	V	T	H	F	Unknown	b
	V	Y	X	K	H	P	V	S	X	Unknown	b
T-cell epitope	R	Y	L	K	D	Q	Q	L	L	HIV gp 41 583–591	c

References:
a: Maier et al. 1994; b: Kubo et al. 1994; c: Dai et al. 1992

Table 2 (Continued)
G HLA-A*3101

	Position											Comments	Re
	1	2	3	4	5	6	7	8	9				
Anchor or auxiliary anchor residues		L V Y F	F L Y W			L F V I			R				a
Other preferred residues	K R	T Q	K N	P D E G S V T	P I V F L Y W	T N D E R	N V R F T H L Y	L R N Q				P1 deduced from individual ligands	
												Source	
Examples for ligands	L	Q	F	P	V	G	R	V	H	R		Histon H2 a 23–32	a
	Q	Q	L	Y	W	S	H	P	R			Ribosomal protein S29 (rat) 3–11	a
	R	G	Y	R	P	R	F	R	R			CCAAT-binding transcription factor 240–248	a
	K	V	F	G	P	I	H	E	R			[GlcNac]-P-transferase 371–379	a
	K	I	M	K	W	N	Y	E	R			Unknown	a
	R	Y	M	D	A	W	N	T	Y	S	R	Lamin B2	a
T-cell epitope	S	T	L	P	E	T	T	V	V	R	R	Hepatitis B cAg 141–151	b

References:
a: Falk et al. 1994c; b: Missale et al. 1993

H HLA-A*3302

	Position											Comments	Ref.
	1	2	3	4	5	6	7	8	9				
Anchor or auxiliary anchor residues		A I L F Y V							R				a
Preferred residues	D E	T	L K	P	P	I L F						P1 deduced from individual ligands	
Other possible residues	M		Q W E N	R D E G S H P	R I F P V L W	R D H Y	H Y V T S	Q N E M					
												Source	
Examples for ligands	D	M	A	A	Q	I	T	Q	R			HLA class I α-chain 161–169	a
	E	S	G	P	S	I	V	H	R			Actin 364–372	a
	T	Y	Y	G	S	F	V	T	R			Unknown	a
	D	Y	I	H	I	R	I	Q	Q	R		Human cDNA HSB15F102 65–74	a
	E	I	M	K	W	N	R	E	R			Unknown	a
	T	I	M	P	K	D	I	Q	L	A	R	Histon 3.1/3.3 118–129	a
T-cell epitope	I	V	G	L	N	K	I	V	R			HIV p24 gag 267–275	b, c

References:
a: Falk et al. 1994c; b: Buseyne et al. 1993; c: Buseyne and Riviere 1993

Table 2 (Continued)
A 1 HLA-A68.1

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues		V T							R K		a
Examples for ligands	A	V	A	A	V	A	A	R	R	Unknown	a
	E	V	A	P	P	E	Y	H	R	Unknown	a
	E	V	A	P	P	E	Y	H	R	K	Unknown	a
	D	V	F	R	D	P	A	L	K		Homologous ribosomal 60S	a
	K	T	G	G	P	I	Y	K	R*		Influenza NP 91–99	a, b
	E	V	I	L	I	D	P	F	H	K	Unknown	a
	T	V	F	D	A	K	R	L	I	G R	HSP 70B / HSC70 66–76	a
	X	V	L	K	X	I	A	K	R*		Unknown	d
	P	V	K	Q	V	V	Y	H	R*		Unknown	d
	E	S	G	P	S	I	V	H	R	K*	β-Actin 364–373	d
	T	T	X	T	T	T	N	A	R*		Unknown	d
	D	T	T	P	T	X	X	R*			Unknown	d
T-cell epitopes	S	T	L	P	E	T	T	V	V	R R	Hepatitis B cAg 141–151	c

* Class I ligands allocated to A68.1 by motif °Also a T-cell epitope

References:
a: Guo et al. 1992; b: Silver et al. 1992; c: Missale et al. 1993; d: Harris et al. 1993

Table 3 HLA-B motifs
A HLA-B7

	Position										Source	Ref.
	1	2	3	4	5	6	7	8	9			
Anchor or auxiliary anchor residues		P	R						L F			a, b
Other preferred residues				D G	D P	F T	L					
Also detected	A H S		D E Q K Y F M N A	E H L K S T P	I V	R L I	V					
Examples for ligands	A	P	R	T	V	A	L	T	A		HLA-DP signal sequence 9–17	a
	A	P	R	T	V	A	L	T	A	L	HLA-DP signal sequence 9–18	a
	A	P	R	A	X	X	X	X	X		Unknown	a
	A	P	R	X	P	X	T	G	X		Unknown	a
	A	P	R	A	S	R	P	S	X		Unknown	a
	A	P	R	T	L	V	L	L	L		HLA-A2.1 signal sequence 5–12	a
	M	P	R	G	V	V	V	T	X		Unknown	a
	S	P	R	Y	I	F	T	M	L		Topoisomerase II 801–809	a
	A	P	A	P	T	V	A	V	X		Unknown	a
	R	P	S	G	P	G	P	E	X		Unknown	a
	L	V	M	A	P	R	T	V	L		HLA-B7 signal sequence 2–10	a
	R	V	M	A	P	R	A	X	X		Unknown	a
	A	P	R	A	F	X	P	X	P	V	Unknown	a
	A	A	S	K	E	R	S	G	V	S L	Histone H1 49–59	a
	A	P	R	S	N	G	M	V	X		Unknown	c
	A	P	R	Q	P	G	X	M	A		Unknown	c
	A	P	A	P	P	P	K	p	M		Ribosomal S26 protein 107–115	c
	A	P	Y	G	G	P	X	A	X		Unknown	c
T-cell epitope	T	P	G	P	G	V	R	Y	P	L	HIV-1 nef 128–137	d

References:
a: Huczko et al. 1993; b: Maier et al. 1994; c: Engelhard 1994; d: Culmann et al. 1991

Table 3 (Continued)
B HLA-B8

	Position 1	2	3	4	5	6	7	8	9	Source	Re a. b
Anchor residues			K		K R				L		
Other preferred residues	G L I		R	E Q D H L S T R G K		N Q H I L Y V E M S T F	E H M N D Q S T Y	E Q H S L V D T	F M		
Examples for ligands	H	P	K	Y	K	T	E	L		Tristetraproline 148–155	d
	I	L	K	Q	K	I	A	d	l	IL-6 precursor 161–169	d
	E	L	K	V	K	N	l	e	l	Restin 1273–1281	d
	E	P	K	Y	K	T	Q	L		Yeast PRAI-SCS 95–102	d
	V	P	K	L	K	V	X	A	L	Rat ribosomal prot. L18, 94–102	d
	F	A	K	P	R	V	G	G		Unknown	d
	S	P	K	L	K	Y	M	Q		Unknown	d
	E	L	K	K	K	T	N	l		Unknown	d
	S	P	K	E	K	I	X	Y		Unknown	d
	X	A	K	E	K	L	A	D		Unknown	d
	S	P	K	E	K	Y	E	X		Unknown	d
	E	L	K	E	K	T	Q	L		Unknown	d
	L	P	K	V	K	L	A	L		Unknown	d
	H	p	K	Y	K	T	E	L		Unknown	d
	V	L	D	L	K	I	V	A	F	Unknown	d
	G	P	K	E	K	X	A	M		Unknown	d
	G	L	K	V	K	G	N	E	F	Unknown	d
	F	L	K	P	K	F	V	A	L	Unknown	d
	E	L	K	I	K	V	Y	X	l	Unknown	d
	S	L	K	E	K	V	X	L		Unknown	d
	E	L	K	E	K	X	y	e	l	Unknown	d
	I	P	K	L	K	N	V	K	r	Unknown	d
	S	L	K	I	K	X	L			Unknown	d
	D	L	K	Q	K	N	E	L		Unknown	d
T-cell epitopes	E	L	R	S	R	Y	W	A	I	Influenza NP 380–388	b
	F	L	R	G	R	A	Y	G	L	EBNA 3 339–347	c
	E	I	Y	K	R	W	I	I	L	HIV gag p24, 262–270	d,
	G	E	I	Y	K	R	W	I	I	HIV gag p24, 261–269	d,
	E	I	K	D	T	K	E	A	L	HIV gag p17, 93–101	d,

References:
a: Malcherek et al. 1993; b: Sutton et al. 1993; c: Burrows et al. 1990; d: DiBrino et al. 1994; e: Phillips et al. 1989; f: Achour et al. 199

Table 3 (Continued)
C HLA-B*2702

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues		R							FYILW		a
Other preferred residues	K		FLX	GPKDEQTS	IKEVMT	IVYRDHEQ	YLVTF	KVDER			
Examples for ligands	SGRKKKGG	RRRRRRR	DLFYKGFF	KTVKKIGK	TKNSALVL	IHVIYTGI	ITVVALNV	MKPKDKRL	WFTYFYYY	F	HGNBPβ-subunit 35–43	a
											Rat ribosomal protein L36 36–44	a
											Human fau protein 114–123	a
											HFPS 191–199	a
											Cytochrome C oxidase 42–50	a
											Actin 63–71	a
											Unknown	a
											Unknown	a

References:
a: Rötzschke et al. 1994

Table 3 (Continued)
D HLA-B*2705

	Position 1	2	3	4	5	6	7	8	9				Source	R
Anchor residues		R							L F					a,
Other preferred residues	A G K R		L I F	K Q E G	I V L P G	I A N Q D V K	I T Y M L W N V P	K N R E Q	Y M I R H K					
Examples for ligands	G	R	L	T	K	H	T	K	F				Rat ribosomal protein L 36 36–44	b
	A	R	L	F	G	I	R	A	K				HBBCP 190–198	a,
	R	R	L	G	X	Q	Y	R	R				Unknown	b
	R	R	F	G	D	K	L	N	F				Immediate early response gene 87–95	b
	G	R	v	F	I	I	K	E					Homologous to Il-1 receptor antagonist	b
	G	R	X	X	i	f	X	r	f	G	Y	H	Unknown	b
	T	R	Y	P	I	L	A	G	H				Cytochrome P450 20–28	b
	r	r	i	s	g	V	D	R	Y				Unknown	a,
	K	R	F	S	F	K	K	S	F				Cattle MARCKS 155–163	b
	A	R	L	Q	T	A	L	L					Rat core histone 188–196	b
	R	R	X	P	I	F	S	R	L				TIS 11B protein 325–333 (X = L)	b
	R	R	F	M	q	Y	Y	V	Y				Homologous to proteasome subunit C5 127–135	b
	R	R	I	K	E	I	V	K	K	H			HSP 86 200–209	b
	R	R	M	G	P	P	V	G	G	H	R		Ribonucleoprotein L 312–322	b
	R	R	I	K	E	I	V	K	K				HSP 89 a 200–208	a
	G	R	I	D	K	P	I	L	K				Ribosomal protein L8 173–181	a
	R	R	S	K	E	I	T	V	R				ATP-dependent RNA helicase 77–85	a
	K	R	F	E	G	L	T	Q	R				Unknown	a
	R	R	V	K	E	V	v	K	k				HSP 89 B 195–203	a
	F	R	Y	N	G	L	I	H	R				60 S ribosomal protein L28 37–45	a
	R	R	Y	Q	K	S	T	E	L				Histon H3.3 52–60	a
	R	R	W	L	P	A	G	d	a				Elongation factor 2 341–349	a
	R	R	I	S	G	V	D	R	Y				Unknown	a
	R	R	P	T	R	P	E	H					Unknown	a
	K	K	Y	Q	K	S	T	E	L				Unknown	c
	XXLNSQDQQCDSSLVE												Draft-1 prooncogene 1–16	i
B*270x-restricted T-cell epitopes	S	R	Y	W	A	I	R	T	R				Influenza NP 383–391	d
	R	R	R	W	R	R	L	T	Y*				EBNA LMP2 236–244	e
	R	R	Y	P	D	A	V	Y	L				Measles F protein 438–446	f
	R	R	I	Y	D	L	I	E	L				EBNA 3C 258–266	e
	K	R	W	I	I	L	G	L	N	K			HIV-1 gag p24 265–274	d,
	G	R	A	F	V	T	I	G	K				HIV-1 gp120 314–322	d
	R	R	K	A	M	F	E	D	I				HSP 60 284–292	h

* B*2704-restricted

References:
a: Jardetzky et al. 1991; b: Rötzschke et al. 1994; c: Shepherd et al. 1993; d: Hunt et al. 1990; e: Brooks et al. 1993; f: van Binnendijk et al. 19
g: Buseyne et al. 1993; h: Corrone et al. 1991; i: Fromento et al. 1993

Table 3 (Continued)

E HLA-B*3501

	Position										Source	Ref.
	1	2	3	4	5	6	7	8	9	10		
Anchor or auxiliary anchor residues		P							Y F M L I	Y		a, b
Other preferred residues	M	A V Y R D	I L F V M E T Y N	K D E G P	D I V T E G L M	I Q K V L M	V N E Q T K	E Q V T				
T-cell epitopes	K K K A	P S P S	K K N R	D D D C	E E K W	L L S V	D D L A	Y Y Y M			P. falciparum CSP 368–375 P. falciparum CSP 368–375 P. falciparum LS 1850–1857 HCV E1 235–242	a a a c

References:
a: Hill et al. 1992; b: Falk et al. 1993b; c: Koziel et al. 1992

F HLA-B*3701

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		D E			V I			F M L	I L		a
Other preferred residues	K Q	H P G S L			T R A D G H M		Q K Y L	T E N D Q G H			
T-cell epitope	E	D	L	R	V	L	S	F	I	Influenza NP 339–347	b

References:
a: Falk et al. 1993b; b: Townsend et al. 1986

G HLA-B*3801

	Position									Source	Ref
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		H	D E						F L		a
Other preferred residues	I	F P W Y	I A S N M V	G E P L V	M T V A E G L K S	V I T K R N H	Y V N	K Y N R T	I		
Examples for ligands	E	H	A	G	V	I	S	V	L	Unknown	a
	T	H	D	E	L	E	D	K	L	Unknown	a
	Q	Y	D	E	A	V	A	Q	F	Histone binding protein 627–635	a
	Y	P	D	P	A	N	G	K	F	Elongation factor 2 265–273	a
	S	H	I	G	D	A	V	V		Cyclin 152–159	a
	Y	H	E	D	I	H	T	Y	L	Cyclin A 178–186	a
	T	F	D	V	A	P	S	R	L	Pm5 protein 270–278	a

References:
a: Falk et al. 1995b

H HLA-B*39011

	Position									Source	Ref
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		R H				I V L			L		a
Other preferred residues			A D I L F V M S T Y	D E G P K	V Y I L P T G K N P	N	N Y F	S K R E T	V I M		
Examples for ligands	S	H	I	G	D	A	V	V		Cyclin 152–159	a
	I	H	E	P	E	P	H	I		CKShs1 protein 59–66	a
	S	R	D	K	T	I	I	M		GBLP 35–42	a

References:
a: Falk et al. 1995b

Table 3 (Continued)

I HLA-B*3902

	Position									Ref.
	1	2	3	4	5	6	7	8	9	
Anchor or auxiliary anchor residues		K Q			I L F V				L	a
Other preferred residues	K A		A I F V N L T Y E H S	G P	N E G P Q S T	V Y T H F I M P R	V L T Y N D H	T S R	F M	

References:
a: Falk et al. 1995b

K HLA-B40*

	Position											Source	Ref.
	1	2	3	4	5	6	7	8	9	10	11		
Anchor or auxiliary anchor residues		E	F I V						L W M A				a
Examples for ligands	T	E	F	P	K	E	R	H	L	R	L	Unknown	a
	G	E	F	P	N	K	N	X	L			Unknown	a
	G	E	F	P	N	K	N	X	L	Y	A	Unknown	a
	G	E	F	P	G	K	I	F	L	Y	A	Unknown	a
	W	E	F	L	Q	P	I	L	L			Unknown	a
	G	E	F	I	P	G	N	D	L	H	R	Unknown	a
	G	E	F	P	P	X	D	N	W			Unknown	a
	E	E	F	Y	V	D	L	E	R			HLA-DQ α 35–41	a
	N	E	F	P	D	I	D	I	R			Unknown	a
	A	E	F	P	K	X	E	A	R			Unknown	a
	A	E	I	G	E	V	I	V	L	W	X	W Unknown	a
	A	E	I	P	G	E	I	A	L			Unknown	a
	G	E	I	L	D	V	P	D	A			IRE-BP 695–703	a
	F	E	I	P	X	L	D	V	A			Unknown	a
	D	E	V	T	P	Q	P	Q	L	V		Unknown	a
	K	E	V	G	V	D	V	A	L	Y	A	Unknown	a
	K	E	S	T	L	H	L	V	L			Ubiquitin 63–71	a
	G	E	V	D	V	E	Q	H	T			Cyclin B 313–321	a
	H	E	E	T	P	P	T	T	S			c-myc 241–249	a

* Motif and ligands deduced by exclusion: Class I ligands from a c-*myc* transfected B-cell line expressing A2, A68, and B40 were sequenced. Those not containing an A2 or A68 motif were thought to contain B40 ligands.

References:
a: Harris et al. 1993

Table 3 (Continued)
L HLA-B*4402

	Position										Ref.
	1	2	3	4	5	6	7	8	9	10	
Anchor or auxiliary anchor residues		E							F Y	F Y	a
Preferred residues	A S		M I L D		I	V	Y				
Others	D		N	P R K							

References:
a: Fleischhauer et al. 1994

M HLA-B*4403

	Position										Source	Ref.
	1	2	3	4	5	6	7	8	9	10		
Anchor or auxiliary anchor residues		E							Y F	Y F		a
Preferred residues	A S		M I L V D									
Others			N	P R K	I V K		Y F					
Examples for ligands	A A	E E	D M	K G	B K	N G	Y S	K F	K K	F Y	HSP 90 427–436 Elongation factor 2 48–57	a a
B*440x-restricted T-cell epitope	E	E	N	L	L	D	F	V	R	F	EBNA 6 130–139	b

References:
a: Fleischhauer et al. 1994; b: Khanna et al. 1992

Table 3 (Continued)

N HLA-B*5101

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		A P G							F I		a
Other preferred residues	I L V Y D	W F	I L M F W Y V E H D R N	G V I K E D	V T G A I S	N I L K Q	K Q R E	T	W M V L		
Examples for ligands	Y D T d l	P A G A P	F H Y Y P	K I L A E	P Y N L V	P L T N N	K N V H R	V H T T Q	I V L L	UBCS, yeast 61–68 Thymidylate synthase 253–261 GBLP 192–200 Unknown Unknown	a a a a a

References:
a: Falk et al. 1995a

O HLA-B*5102

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		P A G	Y						I V		a
Other preferred residues			F V L I	G E K L T Q R N H	V Q N G T	I N Q T	R E Q K	T R Y			
Examples for ligands	Y Y L L T F F M	A P P P G A P P	Y F P P Y Y S W	D K G T L D E F	G P R V N G I K	K P I I T K V G	D K I L V D G w	Y V K v T Y K K	I X V I R V	MHC I α chain 140–148 UBCS, yeast 61–68 Unknown CDC25 homol. 560–567 GBLP 192–200 MHC I α chain 140–148 Ribosomal protein S7/S8A 135–144 Elongation factor 1a 208–216	a a a a a a a a

References:
a: Falk et al. 1995a

Table 3 (Continued)
P HLA-B*5103

	Position									Comments	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		A P G	Y						V I F	Anchor at 9 deduced from individual ligands	a
Other preferred residues	T V D	F W	F D L	E L N R G Q T V	G A V N Q M R	I K T	V M				
Examples for ligands	T D Y	G A F	Y H D	L I d	N Y t	T L L	V N E	T H D	V I F	Source GBLP 192–199 Thymidilate synthase 253–261 Unknown	 a a a

References:
a: Falk et al. 1995a

Q HLA-B*5201

	Position									Comments	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		Q	F Y W		L I V			I V	I V	C-terminal anchor at 8 or 9	a
Other preferred residues	V L I	M F P	I L P D K	L I V P K E A	M F A T G	K N L T S	K E Q Y	M F	M F		
Examples for ligands	T G H G V Y L H	G Q S F Q P Q M	Y F T Y I D F Y	L K I P F P P I	N T M G G A V F	T Y P S N N G L	V A R I K G R H	T I L E M K I T	V V F V	Source GBLP 192–200 Ribos. prot. S21 60–67 P1-CDC21 259–266 MHC II β chain 150–158 RBAP-2 266–273 Elongation factor 2 265–273 Histone 2a Z 25–32 Unknown	 a a a a a a a a

References:
a: Falk et al. 1995a

Table 3 (Continued)

R HLA-B53

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor residues		P									a
T-cell epitope	K	P	I	V	Q	Y	D	N	F	P. falciparum LSA-1 1786–1794	a

References:
a: Hill et al. 1992

S HLA-B*5801

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		A S T		P E K	V I L M F				F W		a
Other preferred residues	K R I	G	G T I L V F Y N Q	D Q R	A D N T Y W Q	I V L F	L Y M N	N R K T	Y		
Examples for ligands	K	A	G	Q	V	V	T	I	W	Lamin C 490–498	a
	A	G	D	R	T	F	Q	K	W	MHC class I 260–268	a
	I	t	T	K	A	I	S	R	F	Unknown	a
	R	T	D	G	K	V	F	Q	F	Ribosomal protein L30 23–31	a
	I	T	S	Q	D	V	L	H	S	W	Cytochrome C oxidase 154–163	a
	I	S	D	S	N	P	F	L	T	Q	Unknown	a
	K	t	D	g	V	V	T	L	F		Unknown	a
	V	T	S	P	L	T	V	E	W		MHC class IIβ 209–217	a
	g	A	V	N	V	V	M	T	f		Glucose transporter 5 322–330	a

References:
a: Falk et al. 1995c

Table 3 (Continued)
T HLA-B60 (B*40012)

	Position									Source	R
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		E					I V		L		a
Other preferred residues			A V I L M F S D N	P K D G N Q T	L I V D T N P G K Q	K N P V I D R Q	L Y M	K R Q			
Examples for ligands	K H Y S I	E E E E E	S A I S V	T T H P D	L L D I P	H R G V D	L c M V T	V w N V K	L A L L E	L m L	Ubiquitin 63–71 MHC class I 221–230 HSHMO2C05 Signal peptidase 45–54 Ribosomal protein S17 95–105	a a a a a

References:
a: Falk et al. 1995c

U HLA-B61 (B*4006)

	Position									Comments	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		E	F I L V Y W			I			V		a
Other preferred residues	G R	P	M T	E G P S N D K A R N Q	V I L M D G V F N S K	N	Y V L W I T R D Q G	K S	A P	P1 deduced from individual ligands	
Examples for ligands	G E G R R G G R	E E E E E E E	F F R I F H M	G Q V R I S G I	G F D D I I L P	F I L N N T i F	G K Y Y A Y I A	S K V V V V K R D	V A V i	Source IEF (mRNA) 9506 127–135 Associated-microfibril. protein 72–80 Ribosomal protein S21 6–13 Ribosomal protein S17 77–84 Ribonucl. reductase 290–297 Ribosomal protein S15 116–123 Unknown Unknown	a a a a a a a a

References:
a: Falk et al. 1995c

Table 3 (Continued)
V HLA-B62 (B*1501)

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		Q L			I V				F Y		a
Other preferred residues	I	M V	K A N F P Y H R	P E G D	G L F T	V T G I	V T L I	Y V T			
Examples for ligands	V	L	K	P	G	M	V	V	T	F	Elongation factor 1 α 271–280	a
	Y	L	G	E	F	S	I	T	Y		Ribosomal protein S15 114–122	a
	G	Q	R	K	G	A	G	S	V		Ribosomal protein L8 (rat) 7–15	a
	K	I	K	S	F	V	K	V	Y		Ribosomal protein L27 66–74	a
	I	Q	P	G	R	G	F	V	L	Y	Unknown	a
	S	Q	F	G	G	G	S	Q	Y		Unknown	a
	G	Q	R	K	P	A	T	S	Y		Ribosomal protein L28 (rat) 68–76	a
	V	Q	G	P	V	G	L				Collagen α1 1106–1112	a
T-cell epitopes	I	L	G	N	K	I	V	R	M	Y	HIV gag 267–276	b

References:
a: Falk et al. 1995c; b: Buseyne et al. 1993

W HLA-B*7801

	Position								Comments	Ref.
	1	2	3	4	5	6	7	8		
Anchor or auxiliary anchor residues		P A G				I L F V		A	This motif is only partial: the C-terminal anchor has not been determined	a
Other preferred residues			Y D W	F D G L V S Q R N	D G V N R Q S T		A V N K Q E	K S		

References:
a: Falk et al. 1995a

Table 4 HLA-C motifs

A HLA-Cw*0301

	Position									Source	
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues			V I Y L M	P		F Y			L F M I		
Other preferred residues		A R	E N	E R	N	M	Q K S M	T			
T-cell epitopes	H or Q	Q M	A V	I H	S Q	P A	R I	T S	L P	R T L	HIV gag 144–152 HIV gag 141–152

References:
a: Falk et al. 1993a; b: Littaua et al. 1991

B HLA-Cw*0401

	Position									Source	
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		Y F				V I L			L F M		a
Other preferred residues			D H	D E P	A H M T R		A	K S H			
T-cell epitope	S	F	N	C	G	G	E	F	F	HIV-1 gp 120 380–388	b

References:
a: Falk et al. 1993a; b: Johnson et al. 1993

C HLA-Cw*0602

	Position									Source	Re
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues					I L F M	V I L			L I V Y		a
Other preferred residues	I F K Y	P R	P I G F Y K N A	P E D Q L	K	A T S	R K Q N	Y E Q N R G T S K			
Examples for ligands	Y V F X	Q R A Q	F H F r	T D p T	G G l P	I G I k	K N q A	K V R g	Y L V I	Y Y	Unknown Unknown Unknown Unknown

References:
a: Falk et al. 1993a

Table 4 (Continued)
D HLA-Cw*0702

	Position									Source	Ref.
	1	2	3	4	5	6	7	8	9		
Anchor or auxiliary anchor residues		Y P			V Y I L F M	V I L M			Y F L		a
Other preferred residues		R D	P G A	D E v Q P S G	T	A R	Y M N R V F E	E A F D K			
Examples for ligands	K	Y	F	D	E	H	Y	E	Y	CKS-2 11–19	a
	R	Y	R	P	G	T	V	A	L	Histone H3.3 40–48	a
	N	K	A	D	V	I	L	K	Y	Protein synthesis factor eIF-4C 87–95	a
	I	Y	P	q	n	v	i	L	Y	Unknown	a
	I	R	K	P	Y	I	w	E	Y	Glutamyl-tRNA synthetase 343–351	a
	N	Y	G	G	G	N	Y	G	S G S Y	Homologous hnRNP A2 or B1 (S11 = N) 277–288	a
	F	Y	P	P	y	l	Y			Unknown	a
	X	M	P	P	f	L	d	G		Unknown	a

References:
a: Falk et al. 1993a

Table 5 Processing motif for all MHC class II ligands

Absolute position																	Ref.
1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	
	P										p	p	p	p	p		a, b, c

References:
a: Falk et al. 1994b; b: Kropshofer et al. 1993; c: Malcherek et al. 1993

Table 6 Human MHC class II motifs
A HLA-DRB1*0101

	Relative position									Source		Ref.
	1	2	3	4	5	6	7	8	9			
Anchor residues	Y,V, L,F, L,A M,W			L,A I,V M,N Q		A,G S,T P			L,A I,V N,F Y			a, b, e
Examples for ligands VGSD	W	R	F	L	R	G	Y	H	Q	YA	HLA-A2 103–117	c
VGSD	W	R	F	L	R	G	Y	H	Q	YAYDG	HLA-A2 103–120	c
VGSD	W	R	F	L	R	G	Y	H	Q	Y	HLA-A2 103–116	c
GSD	W	R	F	L	R	G	Y	H	Q	YA	HLA-A2 104–117	c
LPKPPKPVSK	M	R	M	A	T	P	L	L	M	QALPM	Invariant chain 97–120	c
IPAD	L	R	I	I	S	A	N	G	C	K	Na^+-K^+-ATPase 199–216	c
RVE	Y	H	F	L	S	P	Y	V	S	PKESP	Transferrin receptor 680–696	c
YKHT	L	N	Q	I	D	S	V	K	V	WPRRPT	Cattle fetuin 56–74	c
AILE	F	R	A	M	A	Q	F	S	R	KTD	Unknown	d
PK	Y	V	K	Q	N	T	L	K	L	AT*	Influenza HA 306–318	e

* Alignment determined by structural analysis
References:
a: Hammer et al. 1992; b: Falk et al. 1994b; c: Chicz et al. 1992; d: Kropshofer et al. 1992; e: Stern et al. 1994

Table 6 (Continued)
B HLA-DRB1*0301 (DR17)

		Relative position									Source		F
		1	2	3	4	5	6	7	8	9			a
Anchor or auxiliary anchor residues		L,I F,M V			D		K,R E,Q N			Y,L F			
Examples for ligands	ISNQ	L	T	L	D	S	N	T	K	Y	FHKLN	Apolipoprotein B 2877–2894	a
	ISNQ	L	T	L	D	S	N	T	K	Y	FHKL	Apolipoprotein B 2877–2893	a
	ISNQ	L	T	L	D	S	N	T	K	Y	FHK	Apolipoprotein B 2877–2892	a
	VDT	F	T	L	D	S	K	N	L	Y	HSEA	α1-Antitrypsin 149–164	a
	KPRA	I	S	E	D	V	V	H	G	F	MY	LDL-Receptor 518–532	a
	KQT	I	I	V	D	P	R	N	M	I		IgG2a. Membrane domain	a
	YPD	F	V	P	D	Y	K	E	K	D	KV	Unknown	a
	NIQ	L	R	M	D	P	E	V	A	R	FD	Unknown	a
	LLS	F	R	N	D	Q	N	Q	Y	R	ADI	Transferrin receptor 618–632	a
	LPKPPKPVSK	M	R	R	A	L	P	L	L	M		Invariant chain 97–113	d
	LPKPPKPVSK	M	R	R	A	T	P	L	L	M	QALP	Invariant chain 97–119	d
	LPKPPKPVSK	M	R	R	A	T	P	L	L	M	QALPM	Invariant chain 97–120	d
	PKPPKPVSK	M	R	R	A	T	P	L	L	L		Invariant chain 98–113	d
	PKPPKPVSK	M	R	R	A	T	P	L	L	L	QA	Invariant chain 98–117	d
	KPPKPVSK	M	R	R	A	T	P	L	L	L	Q	Invariant chain 99–116	d
	KPPKPVSK	M	R	R	A	T	P	L	L	L	QALPM	Invariant chain 99–119	d
	VDDTQF	V	R	R	A	T	P	L	L	M	Q	HLA-A30 28–?	e
	ATKYGN	M	R	R	A	T	P	L	L	M	LQNA	Invariant chain 131–149	e
	VFLL	L	R	R	A	T	P	L	L	M	SLS	ACh receptor 289–304	e
	LNK	I	R	R	A	T	P	L	L	M	K	ICAM-2 64–76	e
	GPPKLD	I	R	R	A	T	P	L	L	M	IDIFH	IFN-γ receptor 128–147	e
	GPPKLD	I	R	R	A	T	P	L	L	M	IDIFHP	IFN-γ receptor 128–148	e
	GKFA	I	R	R	A	T	P	L	L	M	IIRTV	Cyt-b5 155–172	e
	YAN	I	K	K	A	T	P	L	L	M	TDMTF	Apolipoprotein B 1207–1224	e
	NLF	L	K	K	A	T	P	L	L	M	TLNKNSLK	Apolipoprotein B 1276–1295	e
	IPDNLF	L	K	K	A	T	P	L	L	M	TLNKN	Apolipoprotein B 1273–1292	e
	IPDNLF	L	K	K	A	T	P	L	L	M	TLNK	Apolipoprotein B 1273–1291	e
	IPDNLF	L	K	K	A	T	P	L	L	M	TLN	Apolipoprotein B 1273–1290	a, e
	IPDNLF	L	K	K	A	T	P	L	L	M	TL	Apolipoprotein B 1273–1289	e
	NLF	L	K	K	A	T	P	L	L	M	TLNK	Apolipoprotein B 1276–1291	e
	NLF	L	K	K	A	T	P	L	L	M	TLN	Apolipoprotein B 1276–1290	e
	VTT	L	K	N	A	T	P	L	L	M	LDLTN	Apolipoprotein B 1294–1810	e
		V	G	S	D	D	G	Y	N	R	GYHQYA	HLA-A2 103–117	e

References:
a: Malcherek et al. 1993; b: Geluk et al. 1994; c: Geluk et al. 1992; d: Riberdy et al. 1992; e: Chicz et al. 1993; f: Sette et al. 1992

Table 6 (Continued)
C HLA-DRB1*0401 (DR4Dw4)

		Relative position									Source		Ref.
		1	2	3	4	5	6	7	8	9			
Anchor or preferred residues		F,Y W,I L,V M			F,W LL V,A D,E no R,K		N,S T,Q H,R	pol.* chg.* ali.*		pol.* ali.* K			a, b, c, d
Examples for ligands		F	V	R	F	D	S	D	A	A	SQRMEP	HLA-A2 33–47	a
	VDDTQ	F	V	R	F	D	S	D	A	A	SQRM	HLA-A2 28–45	a
		F	V	R	F	D	S	D	A	A	SQRM	HLA-A2 33–45	a
	VDDTQ	F	V	R	F	D	S	D	A	A	SPRGEP...	HLA-C 28–?	a
	DGKD	Y	I	A	L	N	E	D	L	S	S	HLA-B44 143–156	a
	LSS	W	T	A	A	D	T	A	A	Q	ITQ	HLA-B44 154–168	a
	LSS	W	T	A	A	D	T	A	A	Q	IT	HLA-B44 154–167	a
	IY	F	R	N	Q	K	G	S	H	S	GLQPTGFL	HLA-DR4β 252–270	a
	DVA	F	V	K	D	Q	T	V	I	Q	NTD	Cattle transferin 68–82	a
	YDHN	F	V	K	A	I	N	A	I	Q	KSW	Cathepsin C 170–185	a
	KHKV	Y	A	C	E	V	T	H	Q	G	...	Igκ chain C region 80–?	a
	HKV	Y	A	C	E	V	T	H	Q	G	L...	Igκ chain C region 81–?	a
	DGP	F	R	I	I	T	V	P	A	A	LDY	Unknown	a
	TGN	Y	R	I	E	S	V	L	S	S		Sphingolipid activator protein 3 165–176	a
	GERA	M	T	K	D	N	N	L	L	G	...	HSC 70 445–?	a
	XXX	Y	E	X	A	L	S	L	P	S	K...	Unknown	a
	GSLF	V	Y	N	I	T	T	N	K	Y	KAFLKQ	VLA-4 229–247	e
	SPEDF	V	Y	Q	F	K	G	M	C	Y	F	HLA-DQβ 3.2 chain 24–38	e
	AAPYEKEVP	L	S	A	L	T	N	I	L	S	AQL	PAJ-1 261–281	e
	GVYF	Y	L	Q	W	G	R	S	T	L	VSVS	Ig heavy chain 121–?	e
	AEALERM	F	L	S	F	P	T	T	K	T		Cattle hemoglobin 26–41	e
	LRS	W	T	A	A	D	T	A	A	Q	ITQRKWEAA	HLA-Cw9 130–150	e
	DLSS	W	T	A	A	D	T	A	A	Q	ITQRKWEAA	HLA-Bw62 129–150	e
	APSP	L	P	E	T	T	E	N	V	V	CALG	HLA-DRα chain 182–198	e

* pol.: Polar; chg.: charged; ali.: aliphatic
References:
a: Friede and co-workers, submitted; b: Sette et al. 1993; c: Hammer et al. 1993; d: Hill et al. 1994; e: Chicz et al. 1993

D HLA-DRB1*0402 (DR4Dw10)

		Relative position									Source		Ref.
		1	2	3	4	5	6	7	8	9			
Anchor or preferred residues		V,I L,M			Y,F W,I L,M R,N no D,E		N,Q S,T K	R,K H,N Q,P; rare D,E		pol.* ali.* H			a
Examples for ligands	GPDGR	L	L	R	G	H	N	Q	F	A	YDGKD	HLA-B38 128–146	a
	GR	L	L	R	G	H	N	Q	F	A	YDGK	HLA-B38 131–145	a
	I	I	K	G	V	R	K	S	N	A	AERRG	HLA-DRα 238–252	a
		I	Y	F	R	N	Q	K	G	H	SGLQPTGFLS	DR4β 248–266	a
				F	R	N	Q	K	G	H	SGLQP	DR4β 250–261	a
	F	I	Y	F	R	N	Q	K	G	H	SGLQPTGFLS	DR4β 249–266	a
		Y	V	R	F	D	S	D	V	G	EY	DR4Dw10β 37–47	a
	LPKPPKPVSK	M	R	M	A	T	P	L	L	Q		Invariant chain 97–?	a
	FDQK	I	V	E	W	D	S	R	K	S	KYFE	BLAST-1 62–78	a
	DQK	I	V	E	W	D	S	R	K	S	KYF	BLAST-1 63–77	a
	IKI	I	S	K	I	E	N	H	E	G	VRR	Pyruvate kinase 264–278	a
	IKI	I	S	K	I	E	N	H	E	G	VR	Pyruvate-kinase 264–277	a
	FGR	I	G	R	L	V	T	R	A	A	FNSG	GAPDH 11–25	a
	FGR	I	G	R	L	V	T	R	A	A	FN	GAPDH 11–23	a
	GFGR	I	G	R	L	V	T	R	A	A	FNSG	GAPDH 10–25	a
	CNE	I	I	N	W	L	D	K	N	Q		HSC 70 574–585	a
	QPD	L	R	Y	L	F	L	N	G	N		Leucine-rich α2-glycoprotein 200–211	a

References:
a: Friede and co-workers, submitted

Table 6 (Continued)

E HLA-DRB1*0404 (DR4Dw14)

		Relative position									Source	
		1	2	3	4	5	6	7	8	9		
Anchor or preferred residues		V,I L,M			F,Y W,I L,V M,A D,E no R,K		N,T S,Q R	pol.* chg.* ali.*		pol.* ali.* K		
Examples for ligands	GSHS	M	R	Y	F	H	T	A	M	S	RPGRGE	HLA-B60 1–?
	SHS	M	R	Y	F	H	T	A	M	S	RPGRGE	HLA-B60 2–?
	YDNS	L	K	I	I	S	N	A	S	C	TTN	GAPDH 139–154

* pol.: Polar; chg.: charged; ali.: aliphatic

References:

a: Friede and co-workers, submitted

F HLA-DRB1*0405 (DR4Dw15)

		Relative position									Source	R
		1	2	3	4	5	6	7	8	9		
Anchor or preferred residues		F,Y W,V L,I M			V,I L,M D,E		N,S T,Q K,D	pol.* chg.* ali.*		D,E Q		a
Examples for ligands	YPTQRAR	Y	Q	W	V	R	C	N	P	D	SNS	PGSG 1–19 a
	QRAR	Y	Q	W	V	R	C	N	P	D	SNS	PGSG 4–19 a
	RAR	Y	Q	W	V	R	C	N	P	D	SNS	PGSG 5–19 a
	KPPQ	Y	I	A	V	H	V	V	P	D	Q	MIF 32–45 a
	FRE	F	K	L	S	K	V	W	R	D	QH	Transferrin receptor 173–186 a
	FRE	F	K	L	S	K	V	W	R	D	Q	Transferrin receptor 173–185 a
	RE	F	K	L	S	K	V	W	R	D	QH	Transferrin receptor 174–186 a
	RE	F	K	L	S	K	V	W	R	D	Q	Transferrin receptor 174–185 a
	VEPDH	Y	V	V	V	G	A	Q	R	D	A	Transferrin receptor 397–411 a
	EPDH	Y	V	V	V	G	A	Q	R	D	A	Transferrin receptor 398–411 a
	THY	Y	A	V	A	V	V	K	K	D	TDFK	Transferrin 92–107 a
	KELK	I	D	I	I	P	N	P	Q	E	R	Hsp 90-beta 68–81 a
	YLL	Y	Y	T	E	F	T	P	T	E	KD	β_2-microglobulin 83–96 a
	LL	Y	Y	T	E	F	T	P	T	E	KDEY	β_2-microglobulin 84–98 a
	CAIHAKR	V	T	I	M	P	K	D	I	Q	LA...	Histone H3 110–? a
	APNT	F	K	T	L	D	S	W	R	D		ras-related protein RAB-7 (rat) 86–98 a
	VADK	I	Q	L	I	N	N	M	L	D		Phosphoglycerate kinase 216–228 a
	GSTV	F	D	N	L	P	N	P	E	I	DGDYYGW	Unknown b
	XXXQ	Y	I	A	V	H	V	V	P	D	QT	Homol. MIF 32–46 b
	SDPIL	Y	R	P	V	A	V	A	L	D		PKM2 99–112 b
		V	P	I	Q	R	A	V	Y	Q	NVVVNNPXD	Unknown b
	SPGTGA	Y	Y	V	L	L	N	...				Unknown b
	KPPQ	Y	I	A	V	H	V	V	P	D	QLM	MIF 32–47 c
	KPPQ	Y	I	A	V	H	V	V	P	D	QL	MIF 32–46 c
	KPPQ	Y	I	A	V	H	V	V	P	D	Q	MIF 32–45 c
	DPIL	Y	R	P	V	A	V	A	L	D	TKGPE	PKM2 101–118 c
	DPIL	Y	R	P	V	A	V	A	L	D	TKGP	PKM2 101–117 c
	DNPQTHY	Y	A	V	A	V	V	K	K	D	TDFKL	Transferrin 88–108 c
	DNPQTHY	Y	A	V	A	V	V	K	K	D	TDFK	Transferrin 88–107 c
	NPQTHY	Y	A	V	A	V	V	K	K	D	TDFKL	Transferrin 89–108 c
	NPQTHY	Y	A	V	A	V	V	K	K	D	TDFK	Transferrin 89–107 c
	DNPQTHY	Y	A	V	A	V	V	K	K	D		Transferrin 88–103 c
	THY	Y	A	V	A	V	V	K	K	D	TDF	Transferrin 92–106 c
	LL	Y	Y	T	E	F	T	P	T	E	KDEY	β_2m 84–98 c
	L	Y	Y	T	E	F	T	P	T	E	KD	β_2m 85–26 c
	XXXXKK	V	V	V	Y	L	Q	K	L	D	T	Cathepsin C 58–73 c
	KK	V	V	V	Y	L	Q	K	L	D	TAYD	Cathepsin C 62–76 c
	K	V	V	V	Y	L	Q	K	L	D	TAYD	Cathepsin C 63–76 c
	KP	Y	N	E	A	K	T	X	F	D	KY	Apolipoprotein B-100 3218–3230 c

* pol.: Polar; chg.: charged; ali.: aliphatic

References:

a: Friede and co-workers, submitted; b: Matsushita et al. 1994; c: Kinouchi et al. 1994

Table 6 (Continued)
G HLA-DRB1*1101

		Relative position									Source	Ref.
		1	2	3	4	5	6	7	8	9		
Anchor residues		W,Y F			M,L V,I		R,K					a, b
Examples for ligands	IDF	Y	T	S	I	T	R	A	R	F	EE	HSC 70 291–305	b
	CPAG	Y	T	C	N	V	K	A	R	S	CEK	Granulin D 41–56	b
	VNH	F	I	A	E	F	K	R	K	H	KKD	Homol. HSC 70 238–252	b
	VNH	F	I	A	E	F	K	R	K	H	K	Homol. HSC 70 238–250	b
	MR	Y	F	H	T	S	V	S	R	P	GRGEP	HLA-Bw61 5–20	b
	KHKV	Y	A	C	E	V	T	H	Q	G	LS	Homol. Ig κ-chain 190–204	b

References:
a: Hammer et al. 1993; b: Newcomb and Cresswell 1993

H HLA-DRB1*1201

		Relative position									Source	Ref.
		1	2	3	4	5	6	7	8	9		
Anchor residues		L,L F,Y V		L,M N,V A			V,Y F,I N,A			Y,F M,I V		a
Examples for ligands	GPDGRL	L	R	G	Y	D	Q	F	A	Y	DGK	HLA-B38 104–121	a
	GPDGRL	L	R	G	H	N	Q	Y	A	Y	D	HLA class I 104–119	a
	TGT	I	K	L	L	N	E	N	S	Y	VP	Transferrin receptor 142–155	a
	T	I	K	L	L	N	E	N	S	Y	VPR	Transferrin receptor 144–156	a
	FTGT	I	K	L	L	N	E	N	S	Y	VPR	Transferrin receptor 141–156	a
	DFTGT	I	K	L	L	N	E	N	S	Y	VPR	Transferrin receptor 140–156	a
	SDEK	I	R	M	N	R	V	V	R	N	NLR	Valosin-cont. protein p97 78–93	a
	SSV	I	T	L	N	T	N	V	G	L	YXQT	Homol. to apolipoprotein	a
	EAL	I	H	Q	L	K	I	N	P	Y	VLS	Unknown	a
	AHL	F	K	Q	N	K	V	V	H	V	NG	Dihydrolipoamide dehydrogenase 138–152	b

References:
a: Falk et al. 1994b; b: Falk and co-workers, unpublished

I HLA-DRB1*1501 (DR2b)

		Relative position									Source	Ref.
		1	2	3	4	5	6	7	8	9		
Anchor residues		L,V I			F,Y I			L,L V,M F				a, b
Examples for ligands	EAEQ	L	R	A	Y	L	D	G	T	G	VE	HLA-A3 152–166	a
		L	E	E	F	G	R	F	A	S	FEAQG	HLA-DRα 45–58	a
	D	V	G	V	Y	R	A	V	T	P	QGRPDA	HLA-DQw6 43–58	a
T-cell epitope	PV	V	H	F	F	K	N	I	V	T		MBP 85–95	b

References:
a: Vogt et al. 1994; b: Wucherpfennig et al. 1994

Table 6 (Continued)

K HLA-DRB5*0101 (DR2a)

		Relative position									Source
		1	2	3	4	5	6	7	8	9	
Anchor or preferred residues		F,Y L,M			Q,V L,M					R,K	
Examples for ligands	DVGV	Y	R	A	V	T	P	Q	G	R	P HLA-DQw6 43–56
	DVGV	Y	R	A	V	T	P	Q	G	R	PDA HLA-DQw6 43–58
	DSDVGV	Y	R	A	V	T	P	Q	G	R	PD HLA-DQw6 41–57
	DSDVGV	Y	R	A	V	T	P	Q	G	R	PDA HLA-DQw6 41–58
	DSDVGV	Y	R	A	V	T	P	Q	G	R	PDAEY HLA-DQw6 41–60
	AAD	M	A	A	Q	I	T	K	R	K	WEAAH HLA-A3 135–151
	TAAD	M	A	A	Q	I	T	K	R	K	WEA HLA-A3 134–149
	DVGE	F	A	A	V	T	E	K	R	R	PDAEYW HLA-DR2b 43–61
T-cell epitopes	PK	Y	V	K	Q	N	T	L	K	L	AT HA 307–319
		L	Q	A	A	P	A	L	D	K	L HSP65 418–427
	VHF	F	K	N	I	V	T	P	R	T	P MBP 87–99
	ASD	Y	K	S	A	H	K	G	F	K	GVD MBP 131–145
	KG	F	K	G	V	D	A	Q	G	T	LSKI MBP 139–153

References:
a: Vogt et al. 1994; b: Wucherpfennig et al. 1994; c: O'Sullivan et al. 1991; d: Anderson et al. 1988; e: Martin et al. 1991

L HLA-DQA1*0501/DQB1*0301

		Relative position									Source
		1	2	3	4	5	6	7	8	9	
Anchor residues		F,Y L,M L,V				V,L L,M Y		Y,F M,L V,I			
Prefered residues	A		A	A	A						
Examples for ligands	TPL	L	M	Q	A	L	P	M	G	A	LPQG Invariant chain 111–126
	TPL	L	M	Q	A	L	P	M	G	A	LPQ Invariant chain 111–125
	KPPKPVSKMR	M	A	T	P	L	L	M	Q	A	Invariant chain 99–117
	LPKPPKPVSKMR	M	A	T	P	L	L	M			Invariant chain 97–115
	IPE	L	N	K	V	A	R	A	A	A	Transferrin receptor 579–597
	DVEV	Y	R	A	V	T	P	L	G	P	EVAGQF DQβ chain 43–55

References:
a: Falk et al. 1994b

M HLA-DPA1*0201/DPB1*0401

		Relative position										Source
		1	2	3	4	5	6	7	8	9	10	
Anchor residues		F,L Y,M I,V A						F,L Y,M V,I A			V,Y I,A L	
Examples for ligands	EKK	Y	F	A	A	T	Q	F	E	P	L	AARL Unknown
	KK	Y	F	A	A	T	Q	F	E	P	L	AARL Unknown
	EKK	Y	F	A	A	T	Q	F	E	P	L	Unknown
	GPG	A	P	A	D	V	Q	Y	D	L	Y	LNVANRR IL-3 Receptor α-chain 127–146

References:
a: Falk et al. 1994b

Table 6 (Continued)
N HLA-DPA1*0102/DPB1*0201

		Relative position									Source		Ref.
		1	2	3	4	5	6	7	8	9			
Anchor residues		F,L M,V W,Y				F,L M,Y			L,A M,V				a
Examples for ligands	ADEKKF	W	G	K	Y	L	Y	E	I	A	RRHP	Cattle serum albumin 152–170	a
	GEP	L	S	Y	T	R	F	S	L	A	RQVDG	Transferrin receptor 15–31	a
	LPSQA	F	E	Y	I	L	Y	N	K	G		Cathepsin H 185–198	a

References:
a: Rötzschke and Falk 1994

Table 7 Other human class II ligands

MHC molecule	Peptide sequence	Source		Ref.
HLA-DR2 (DRB5*0101 or DRB1*1501)	NIVIKRSNSTAATNEVPEVTVFS	HLA-DQα	97– 119	a
	NIVIKRSNSTAATNEV	HLA-DQα	97– 112	a
	SDVGVYRAVTPQGRPDAE	HLA-DQβ	42– 59	a
	DVGVYRAVTPQGRPDAE	HLA-DQβ	43– 59	
	DVGVYRAVTPQGRPD	HLA-DQβ	43– 57	
	RVQPKVTVYPSKTQPLQH	HLA-DRB1*1501	94– 111	a
	RVQPKVTVYPSKTQP	HLA-DRB1*1501	94– 108	a
	LSPIHIALNFSLDPQAPVDSHGLRPALHYQ	Fibronectin receptor α	586– 616	a
	DGILYYYQSGGRLRRPVN	K+ channel protein	173– 190	a
	IQNLIKEEAFLGITDEKTEG	Mannose binding protein	174– 193	a
	EHHIFLGATNYIYVLNEEDLQKV	MET protooncogene	59– 81	a
	QELKNKYYQVPRKGIQA	Guanylate binding protein 2	434– 450	a
	FPKSLHTYANILLDRRVPQTD	Apolipoprotein B100	1200–1220	a
	FPKSLHTYANILLDRRVPQ	Apolipoprotein B100	1200–1218	a
	LWDYGMSSSPHVLRNR	Factor VIII	1775–1790	a
HLA-DRB1*0701	RPAGDGTFQKWASVVVPSGQ	HLA-A29	234– 253	a
	RPAGDGTFQKWASVVV		234– 249	a
	GDGTFQKWASVVVPSGQEQRYT		237– 258	a
	GDGTFQKWASVVVPSGQE		237– 254	a
	GTFQKWASVVVPSG		239– 252	a
	GTFQKWASVVVPSGQ		239– 253	a
	GTFQKWASVVVPSGQEQRYTCHV		239– 261	a
	RETQISKTNTQTYRENL	HLA-B44	83– 99	a
	RETQISKTNTQTYREN		83– 98	a
	RETQISKTNTQTYRE		83– 97	a
	RSNYTPITNPPEVTVLTNSPVELREP	HLA-DR α chain	101– 126	a
	GALANIAVDKANLEIMTKRSN		58– 78	a
	SLQSPITVEWRAQSESAQSKMLSGIGGFVL	HLA-DQ α chain	179–?	a
	VTQYLNATGNRWCSWSLSQAR	4F2	318– 338	a
	VTQYLNATGNRWCSWSL		318– 334	a
	TSILCYRKREWIK	LIF receptor	854– 866	a
	PAFRFTREAAQDCEV	Thromboxane-A synthase	406– 420	a
	GDMYPKTWSGMLVGALCALAGVLTI	K+ channel protein	492– 516	a
	TPSYVAPTDTERLIGDA	Hsp 70	38– 54	a
	TPSYVAFTDTERLIG		38– 52	a
	VPGLYSPCRAFFNKEELL	EBV MCP	1264–1282	a
	VPGLYSPCRAFFNK		1264–1277	a
	KVDLTFSKQHALLCSDYQADYES	Apolipoprotein B 100	1586–1608	a
	KVDLTFSKQHALLCS		1586–1600	a
	FSHDYRGSTSHRL		1942–1954	a
	LPKYFEKKRNTII		2077–2089	a
	APVLISQKLSPIYNLVPVK	Complement C9	465– 483	a
	VGSDWRFLRGYHQYAYDG	HLA-A2	103– 120	a
	PKPPKPVSKMRMATPLLMQALP	Invariant chain	98– 119	a
	APSPLPETTENVVCALGLTV	HLA-DRα chain	182– 200	a
	KHKVYACEVTHQGL	Ig kappa chain	188– 201	a

Table 7 (Continued)

MHC molecule	Peptide sequence	Source		
HLA-DRB1*0801	APSPLPETTENVVCALG	HLA-DRα chain	182– 198	a
	SETVFLPREDHLFRKFHYLPFLP	HLA-DR α chain	158– 180	a
	RHNYELDEAVTLQ	HLA-DP β chain	80– 92	a
	DPQSGALYISKVQKEDNSTYI	LAM Blast-1	88– 108	a
	GALYISKVQKEDNSTYI		92– 108	a
	DPVPKPVIKIEKIEDMDD		129– 146	a
	DPVPKPVIKIEKIED		129– 143	a
	FTFTISRLEPEDFAVYYC	Ig κ chain	63– 80	a
	FTFTISRLEPEDFAV		63– 77	a
	DPVEMRRLNYQTPG	LAR	1302–1316	a
	YQLLRSMIGYIEELAPIV	LIF receptor	709– 726	a
	GNHLYKWKQIPDCENVK	IFN-α receptor	271– 287	a
	LPFFLFRQAYHPNNSSPVCY	IL-8 receptor	169– 188	a
	RPSMLQHLLR	Ca^{2+} release channel	2614–2623	a
	DDFMGQLLNGRVLFPVNLQLGA	CD35	359– 380	a
	IPRLQKIWKNYLSMNKY	CD75	106– 122	a
	EPFLYILGKSRVLZAQ	Calcitonin receptor	38– 53	a
	NRSEEFLIAGKLQDGLLH	TIMP-1	101– 118	a
	RSEEFLIAGKLQDGLL		102– 117	a
	SZEEFLIAGKLQDGLL		103– 117	a
	NRSEEFLIAGKL		101– 112	a
	QAKFPACIKRSDGSCAWYRGAAPPKQEF	TIMP-2	187– 214	a
	QAKFPACIKRSDGSCAWYR		187– 205	a
	DRPFLFVVRHNPTGTVLFM	PAI-1	378– 396	a
	MPHFFRLFRSTVKQVD		133– 148	a
	QNFTVIFDTGSSNLWVPSVYCTSP	Cathepsin E	89– 112	a
	QNFTVIFDTGSSNLWV		89– 104	a
	TAFQYIIDNKGIDSDAS	Cathepsin S	189– 205	a
	DEYYRRLLRVLRAREQIV	Cystatin SN	41– 58	a
	EAIYDICRRNLDIERPT	Tubulin α-1 chain	207– 223	a
	EAIYDICRRNLDI		207– 219	a
	HELEKIKKQVEQEKCEIQAAL	Myosin β heavy chain	1027–1047	a
	AEVYHDVAASEFF...	α-enolase	23–?	a
	KRSFFALRDQIPDL	c-*myc*	371– 385	a
	RQYRLKKISKEERTPGC	K-ras	164– 180	a
	KNIFHFKVNQEGLKLSNDMM	Apolipoprotein B-100	1724–1743	a
	KNIFHFKVNQEGLKLS		1724–1739	a
	YKQTVSLDIQPYSLVTTLNS		1780–1799	a
	STPEFTILNTLHIPSFT		2646–2662	a
	TPEFTILNTLHIPSFTID		2647–2664	a
	TPEFTILNTLHIPSFT		2647–2662	a
	SNTKYFHKLNIPQLDF		2885–2900	a
	LPFFKFLPKYFEKRNT		2072–2088	a
	LPFFKFLPKYFEKKR		2072–2086	a
	WNFYYSPQSSPDKKL.		4022–4036	a
	DVIWELLNHAQEHFGKDKSKE	Cattle transferrin	261– 281	a
	DVIWELLNHAQEHFG		261– 275	a
	DVIWELLNHAQEH		261– 273	a
	IALLLMASQEPQRMSRNFVR	von Willebrand factor	617– 636	a
	IALLLMASQEPQRM		617– 630	a
HLA-DR11 or Dw52	SXVITLNTNVGLYXQS	Homol. Apolipoprotein	3345–3360	b
	DPXQDELQKLNAXDP	Unknown		b
	XPELNKVARAAAEVAG	Homol. Transferrin receptor	580– 595	b
DR17 or DRw 52	TFDEIASGFRQGGASQ	Glucose transporter	459– 474	a
	YGYTSYDTFSWAFL	Na+ channel protein	384– 397	a
	GQVKKNNHQEDKIE	CD45	1071–1084	a
	TGHGARTSTEPTTDY	EBV gp220	592– 606	a
	KELKRQYEKKLRQ	EBV tegument p140	1395–1407	a
	SPLQALDFPGNGPPVNYKTGNL	IP 30	38– 59	a

References:
a: Chicz et al. 1993; b: Newcomb and Cresswell 1993

Table 8 Mouse class II motifs

A H-2E[k]

		Relative position									Source	Ref.
		1	2	3	4	5	6	7	8	9		
Anchor or preferred residues		LL V,F Y,W			LL V,F S		Q,N A			K,R		a, b, c
Examples for ligands	HPPHIE	I	Q	M	L	K	N	G	K	K		c
	DNRM	V	H	F	I	A	E	F	K	R	K	c
	TPTL	V	E	A	A	R	N	L	G	R	VG	c
	VNKE	I	Q	N	A	V	Q	G	V	K	HI	c
	GFPT	I	Y	F	S	P	A	N	K	K	L	a
	IP	L	I	M	L	I	N	K	A	R	NKAE	a
	YDRN	T	K	S	P	L	F	V	G	K	V	a
		F	A	E	F	G	T	L	K	K	AAVHYDRSG	a
	LH	L	G	Y	L	P	N	Q	L	F	R	a
	IPGGP	V	R	L	C	P	G	R	I	R		a
T-cell epitopes	RADL	I	A	Y	L	K	Q	A	T	K		b
	RADL	I	A	Y	L	K	Q	A	T	A	K	b
	LEDARR	L	K	A	I	Y	E	K	K	K		e
	QD	I	L	I	R	L	F	K	S	H	PETL	e
	VTV	L	T	A	L	G	A	I	L	K	K	d
		L	T	A	L	G	G	I	L	K		b
		L	T	A	L	G	T	I	L	K		b
		I	T	A	F	N	E	G	L	K		b
	KVFGR	C	E	L	A	A	A	M	K	R	HGLD	HEL 1–18 e
	SALLSSD	I	T	A	S	V	N	C	A	K		HEL 81–96 d
		W	V	A	W	R	N	R	C	K	GTD	HEL 108–119 d
	VEK	Y	G	P	E	A	S	A	F	T	KKMVENAK	SNase 51–70 e
	RTDKYGRG	L	A	Y	I	Y	A	D	G	K	MVN	SNase 81–100 e
	HEHQ	L	R	K	S	E	A	Q	A	K	KEKLNIW	SNase 121–140 f
		I	A	K	F	G	T	A	F	K		LLO 218–226 b

References:
a: Schild and co-workers, submitted; b: Reay et al. 1994; c: Marrack et al. 1993; d: Spouge et al. 1987; e: Almvia et al. 1994; f: Sette et al. 1989

B H-2E[d]

		Relative position									Source	Ref.
		1	2	3	4	5	6	7	8	9		
Anchor or preferred residues		W,Y F,L L,V			K,R I		LL V,G			K,R		a
Examples for ligands	SQLELR	W	K	S	R	H	I	K	E	R	IL-2R γ chain 168–182	a
	LELR	W	K	S	R	H	I	K	E	R	IL-2R γ chain 170–182	a
	ERAEA	W	R	Q	K	L	H	G	R	L	Apo-E prec. 222–236	a
	RAEA	W	R	Q	K	L	H	G	R	L	Apo-E prec. 223–236	a
	AQ	F	M	W	I	I	R	K	R	I	QLP Unknown	a
	SLDEH	Y	H	I	R	V	H	L	V	K	Similar Apolipoprotein B 2211–2224	a
	GQFY	F	L	I	R	K	R	I	H	L	R C. elegans cDNA homol. 74–87	a
	LV	V	D	N	G	S	G	M	C	K	AGF Actin B 8–21	a
T-cell epitopes	ALWFRNH	F	V	F	G	G	G	T	K	V	TV Ig lambda 91–108	b
	KYLEFISEA	I	I	H	V	L	H	S	R		SWM 102–118	c
	NKALE	L	F	R	K	D	I	A	A	K	Y SWM 132–146	d
	W	V	A	W	R	N	R	C	K	G	TD HEL 108–119	c
	A	Y	V	Y	K	P	N	N	T	H	EQHLRKSE SNase 112–129	e
	SS	F	E	R	F	E	I	F	P	K	FLU PR/8 HA 109–119	c
	LEDARR	L	K	A	I	Y	E	K	K	K	λrep 12–26	c
	EK	I	R	L	R	P	G	G	K	K	K HIV-1 gag p17 17–28	f

References:
a: Schild and co-workers, submitted; b: Bogen et al. 1986; c: Spouge et al. 1987; d: O'Sullivan et al. 1991; e: Chicz et al. 1992; f: Sette et al. 1989

Table 8 (Continued)

C H-2E^b

		Relative position									Comments
		1	2	3	4	5	6	7	8	9	
Anchor or preferred residues		L,V L			L,I V		Q,N			K,R	This motif has been predicted based on prediction of pocket structure and comparison with H-2E^k and H-2E^d motifs
											Source
Examples for ligands		L	Y	V	L	K	I	G	K	K	DG Carboxypeptidase A 44–54
	HPPHIE	I	Q	M	L	K	N	G	K	K	β_2 42–56
	EGEC	V	E	W	L	H	R	Y	L	K	NG H-2L^d 160–174
	MQKETTA	L	A	P	S	T	M	K	I	K	II β-actin 286–303
	CT	F	A	I	C	W	L	P	F	H	VFFL Substance P receptor 255–269
	EGSLI	V	E	K	I	M	Q	S	S	S	E HSP60 478–492
T-cell epitope	DL	I	A	Y	L	K	Q	A	T	K	MCC 93–103

References:
a: Schild and wo-workers, submitted; b: Maffack et al. 1993; c: Alturvia et al. 1994; d: Reay et al. 1994

D H-2E^b

		Relative position									Comments
		1	2	3	4	5	6	7	8	9	
Anchor or preferred residues		W,F Y			L,I F,V		Q,N, A			K,R	This motif has been predicted based on prediction of pocket structure and comparison with H-2E^k and H-2E^b motifs
											Source
Examples for ligands	SPSYV	Y	H	Q	F	E	R	R	A	K	YK MuLV env protein 454–469
	SPSYV	Y	H	Q	F	E	R	R	A	K	YKREPVSL MuLV env protein 454–475
	SPSYV	Y	H	Q	F	E	R	R	A	K	MuLV env protein 454–467
	GK	Y	L	Y	E	I	A	R	R	H	PYFY BSA 141–155
	XPQS	Y	L	I	H	E	X	X	X	I	S Unknown
T-cell epitopes	RTDKYGRG	L	A	Y	I	Y	A	D	G	K	MVN SNase 81–100
	DL	I	A	Y	L	K	Q	A	T	K	MCC 93–103

References:
a: Schild and co-workers, submitted; b: Rudensky et al. 1991; c: Alturvia et al. 1994; d: Reay et al. 1994

Table 9 Other mouse class II ligands

MHC Molecule	Peptide sequence	Source		Ref.
H-2A^a	HNEGFYVCPGPHRP	MuLV env	145–158	a
	ASFEAQGALANIAVDKA	H-2Eα	52– 68	a
	KPVSQMRMATPLLMR	Invariant chain	86–100	a
	NYNAYNATPATLAVD	Unknown		a, b
	RPDAEYWNSQPE	H-2Aβ	55– 66	b
	XNADFKTPATLTVDKP	IgG V_a	59– 74	b
H-2A^b	IRLKITDSGPRVPIGPN	MuLV env	255–269	b
	IRLKITDSGPRVPIG	MuLV env	255–267	b
	WQSQSITCNVAHPASST	IgG2a	194–210	b
	NVEVHTAQTQTHREDY	IgG2a	281–296	b
	KPTEVSGKLVHANFGT	Transferrin receptor	203–218	b
	XPYMFADKVVHLPGSQ	Unknown		b
H-2A^d	WANLMEKIQASVATNPI	Apo-E	268–284	c
	WANLMEKIQASVATNP	Apo-E	268–283	c
	DAYHSRAIQVVRARKQ	Cys-C	40– 55	c
	ASFEAQGALANIAVDKA	H-2I-Eα^d	52– 68	c
	ASFEAQGALANIAVDK	H-2I-Eα ^{96}	52– 67	c
	EEQTQQIRLQAEIFQAR	Apo-E	236–252	c
	EQTQQIRLQAEIFQAR	Apo-E	237–252	c
	KPVSQMRMATPLLMRPM	Li	85–101	c
	VPQLNQMVRTAAEVAGQX	Tf recp.	442–459	c
	ISQAVHAAHAEINE	Ovalbumin	323–336	c
	LEDARRLKAIYEKKK	λ repressor	12– 26	c
H-2A^k	DGSTDYGILQINSR	Hen egg lysozyme	48– 61	d
	DGSTDYGILQINS		48– 60	d
	DGSTDYGILQINSRW		48– 62	d
	DYGILQINSRWW (C)		52– 63 (64)	d
	IIANDQGNRTTPSY	hsp70	28– 41	d
	TPRRGEVYTCHVEHP	H-2I-A^k β chain	165–179	d
	KVHGSLARAGKVRGQTPKVAKQ	S30 ribosomal protein	75– 96	d
	AGKVRGQTPKVAKQEKKKKKT		83–103	d
	EPLVPLDNHIPENAQPG	Ryudocan	84–100	d
	XQLGAQNEMLXPL	Unknown		e
	XXKKGTDFQLNQLE	Transferrin	100–113	e
	KGTDFQLNQLEGKKG	Transferrin	103–117	e
	YVRFDSFVGEYRAVT	H-2Aβ^k	37– 51	e
	XPLALQFAELPVNKG	Unknown		e
	XNLRFDSDVGEFRAV	H-2Eβ^k	33– 47	e
	EDENLYEGLNLDDXSMYE	MBI	177–194	e
	XXLYNKGIMGEdSYPY	Carbepsin H	77– 92	e
	SYLDAXVXEQLAT	Fce-Receptor II	298–310	e
	XXXHFVHQFCPFcyF	H-2Aβ^k	3– 17	e
	QFQPFXYFTNT	H-2Aβ^k	10– 20	e
H-2A^{k7}	KPKATAEQLKTVMDD	Serum albumin	560–574	f
	GHNYVTAIRNQQEG	Transferrin	55– 68	f
	ETTEESLRNYYEQ	hnRNP B1 & A2	31– 43	f
	VVMRDPASKRSRGFGF	hnRNP A2 & B1	51– 66	f
	VVMRDPQTKRSRGFGF	hnRNP A1	44– 59	f
	PKEPEQLRKLFIGGL	hnRNP A1	7– 21	f
	VVYPWTQRYFDSF	β Globin major	33– 45	f

References:
a: Rudensky et al. 1991; b: Rudensky et al. 1992; c: Hunt et al. 1992b; d: Nelson et al. 1992; e: Marrack et al. 1993; f: Reich et al. 1994

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